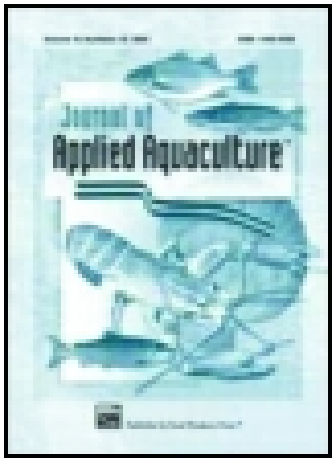

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Effects of Salinity on *Streptococcus* Infection of Nile Tilapia, *Oreochromis niloticus*

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ABSTRACT. The effect of 0, 15, and 30 parts per thousand (ppt) salinities on three isolates of *Streptococcus* infection of "injured" Nile tilapia, *Oreochromis niloticus*, at 25 and 30°C were determined. No deaths occurred in fish in any environmental regimen unless they were exposed to *Streptococcus*. However, increased susceptibility to *Streptococcus* was associated with elevated salinities at 25 and 30°C. Mortalities were not significantly ($P > 0.05$) higher in 15 ppt salinity than 0 ppt at 25°C, but at this temperature, mortalities of *Streptococcus* infected tilapia were significantly ($P < 0.05$) higher at 30 ppt than at 0 or 15 ppt salinities. At 30°C, mortalities of fish infected with two *Streptococcus* isolates were significantly ($P < 0.05$) higher at 15 ppt than an 0 ppt salinities. All groups of fish at 30°C in 0 ppt or 15 ppt salinities had significantly higher mortalities than the corresponding groups held at 25°C. Mean day-to-death generally decreased as temperature was increased from 25 to 30°C, but a greater difference was noted in most instances as salinity increased from 0 ppt to 15 ppt and 30 ppt. *[Article copies available from The Haworth Document Delivery Service: 1-800-342-9678. E-mail address: getinfo@haworth.com]*

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INTRODUCTION

Changes in water quality may be directly lethal to fish, and sublethal changes may stress fish sufficiently to predispose them to infectious diseases (Snieszko 1974; Wedemeyer and Wood 1974). Walters and Plumb (1980) demonstrated that poor water quality involving low dissolved oxygen, elevated carbon dioxide, and elevated ammonia levels predisposed channel catfish, *Ictalurus punctatus*, to bacterial infection. The frequency of fish disease is also influenced by water temperature (Moller 1985). For example, a high rate of "red spot disease" in striped mullet, *Mugil cephalus*, occurred following a rapidly changing water temperature and salinity (Rodgers and Burke 1981).

Streptococcus sp. infects fish most often in waters with elevated salinities but may occasionally occur in freshwater fish (Plumb 1991). Pathogenicity of this bacterium in freshwater fish has been reported to differ from that of marine fish (Ohnishi and Jo 1986). *Streptococcus* sp. is not an obligate fish pathogen, because it can be present in the environment without causing fish health problems. As indicated by Plumb et al. (1974) stress may predispose fish to infections. In freshwater fish, *Streptococcus* infections frequently occur at temperatures above 25°C (Jo 1982); however, the relationship between the bacterium, fish, and environment remains unclear.

Outbreaks of *Streptococcus* in Nile tilapia, *Oreochromis niloticus*, have been reported in Japan, Taiwan, and the United States (Kitao et al. 1981; Miyazaki et al., 1984; Tong et al. 1985). As an euryhaline fish, Nile tilapia can adapt to either fresh, brackish, or sea water (Suresh and Lin 1992). Therefore, the objective of this study was to determine the pathogenicity of *Streptococcus* sp. in Nile tilapia at various combinations of two temperatures and three salinity concentrations.

MATERIALS AND METHODS

Three isolates of *Streptococcus* sp. from fish in the United States were used. Isolate MS91-452 was taken from channel catfish in Mississippi in 1991; isolate Lake was from speckled trout, *Cynoscion nebulosus*, in Lake Pontchartrain, Louisiana in 1979; and isolate DL805 was taken from gulf killifish, *Fundulus grandis*, in Alabama in 1980. These streptococci were passed through Nile tilapia three times to enhance virulence. After these passages, a fresh isolate was then cultured on brain heart infusion (BHI) agar at 30°C, harvested 24 hours later, and suspended in 0.85% saline solution. The bacterial concentration was determined by serial dilution of the culture, using BHI pour plates, and calculating the colony-forming

units (CFU). Bacterial density in the dilutions were calibrated spectrophotometrically for subsequent CFU determinations.

Nile tilapia, averaging 20 g each, were obtained from the Auburn University Fisheries Research Station, Auburn, Alabama. Fish were divided into three groups and acclimated for 10 days in 200-L (25°C) fiberglass troughs filled with water having 0, 15, or 30 ppt salinity reconstituted with Super Salt Concentration (Fritz Chemical Co., Dallas, Texas[1]). Salinity was measured with a refractometer and adjusted to the desired concentration by additional sea salts. Fish were transferred directly from freshwater to 15 ppt salinity. For the 30 ppt salinity studies, fish were gradually acclimated from 15 ppt to 30 ppt over a 2-week period by increasing salinity 3 ppt every 2 days. During this period, fish were fed a commercially pelleted catfish ration at a rate of 1% of their body weight per day.

Experiments were carried out in 50-L glass aquaria containing 40 L of static 0, 15, or 30 ppt salinity water. Aquaria were aerated by water recirculating through in-tank, charcoal-glass wool filters; temperature was adjusted to 25 or 30°C with submersible aquarium heaters.

Streptococci were cultured as previously described, centrifuged at 15,000 × g for 20 minutes, and resuspended in 1 L of 0.85% saline solution. The bacterial suspension was added to a water bath to provide approximately 2×10^6 CFU/mL based on previous plate counts and appropriate optical density. Just prior to exposure to the bacteria, fish were removed from aquaria and "injured" by scraping approximately 1.5 cm^2 of the lateral body surface with a scalpel. Exposure was by immersion of 10 fish in the bacterial suspension for 10 minutes, and then returning the fish to their respective aquaria. Three replicates of 10 fish each were used in each temperature (25 or 30°C) and salinity (0, 15, and 30 ppt) regimen for each *Streptococcus* isolate. As controls, three aquaria of 10 injured fish with no exposure to the bacteria were set up for each temperature and salinity.

Daily observations were made for 14 days. The cumulative mortality for each treatment was analyzed and compared by analysis of variance (SAS 1982). Probability values of 0.05 or less were considered statistically significant. The mean day-to-death was calculated by averaging the length of time (days) from exposure to *Streptococcus* to death of the animals in a given treatment.

RESULTS AND DISCUSSION

Nile tilapia infected with *Streptococcus* showed erratic swimming, spiraling, and/or loss of equilibrium before dying, regardless of tempera-

1. Use of trade or manufacturer's name does not imply endorsement.

ture or salinity. No disease or mortality occurred in injured control fish at either 25 or 30°C in 0, 15, or 30 ppt salinity. Among the infected-injured groups at 25°C and 0 ppt salinity there were 27, 33, and 0% mortality in fish exposed to isolates Lake, DL805, and MS91-452, respectively (Table 1). Significantly higher mortality occurred in fish infected with each isolate at 30°C than in those infected at 25°C in 0 and 15 ppt salinity. At 30°C and 15 ppt salinity, the mortality was 100, 100, and 63% for isolates Lake, DL805, and MS91-452, respectively. Rasheed and Plumb (1984) reported a higher mortality of gulf killifish at 30°C and concluded that the growth rate of *Streptococcus* in fish is similar to that *in vitro*. In view of this, more rapid growth of bacteria at 30°C may contribute to high mortality in fish. Virulence of the pathogen may also affect the bactericidal activity of the host defense mechanism. Secombes and Fletcher (1992) proposed that the more virulent pathogen is less likely to be killed by phagocytosis. Also, some strains of Group B *Streptococcus* appear more virulent than others in experimental animals, due to their high resistance to opsonization (Santos et al. 1982).

Nile tilapia naturally inhabits freshwater; however, it can adapt to higher salinities (Morgan and Iwama 1991; Suresh and Lin 1992). In our study, increased salinity adversely affected the mortality of fish in a given temperature group when those fish were exposed to *Streptococcus* (Table 1). As salinity increased from 0 to 15 ppt at a particular water temperature, mortality from all isolates were higher, but they were not significantly different ($P > 0.05$). At 25°C, as salinity increased from 15 ppt to 30 ppt, a significant increase ($P < 0.05$) in mortality occurred in all groups exposed to each

TABLE 1. Mortality (%) of injured Nile tilapia infected with three isolates of *Streptococcus* and held at two temperatures and three salinities. Each treatment consisted of three aquaria with 10 fish each. Values within columns with different letters are significantly ($P < 0.05$) different.

Temp.	Salinity	Infected (isolate)			
(°C)	(ppt)	Lake	DL805	MS91-452	Non-infected
25	0	27 ± 2a	33 ± 5a	0 ± 0a	0
25	15	40 ± 3a	43 ± 7a	0 ± 0a	0
25	30	63 ± 2b	73 ± 2b	40 ± 3b	0
30	0	73 ± 8b	100 ± 0b	3 ± 2a	0
30	15	100 ± 0b	100 ± 0b	63 ± 5c	0

TABLE 2. Mean days-to-death of Nile tilapia, infected by immersion with three isolates of *Streptococcus* and held at two temperatures and three concentrations of salinity.

Temp.	Salinity	Mean days-to-death/isolate		
(0°C)	(ppt)	Lake	DL805	MS91-452
25	0	10.0	11.2	No deaths
25	15	6.3	11.2	No deaths
25	30	4.9	7.6	6.0
30	0	7.4	5.6	8.0
30	15	6.0	4.6	8.5

isolate. At 30°C, the same trend occurred in infected fish when salinity was increased from 0 to 15 ppt. According to Al-Amoudi (1987) Nile tilapia can adapt immediately from fresh water to 18 ppt, but a gradual acclimation is required for transfer to higher salinity without mortality. This was borne out in our study, because no losses occurred in the 15 or 30 ppt salinity unless the fish had been exposed to *Streptococcus*. When water temperature increased from 25 to 30°C at 0 ppt salinity, a significant difference in mortality occurred in fish exposed to Lake and DL805 isolates (Table 1).

By comparing the mean days-to-death, the virulence of the Lake and DL805 isolates was similar, but strain MS91-452 was less virulent (Table 2). However, the increased temperature and salinity in most instances decreased the mean days-to-death following challenge with *Streptococcus*. The shortest mean days-to-death for each isolate was 4.9 days at 25°C in 30 ppt salinity for isolate Lake, 4.5 days at 30°C in 15 ppt salinity for DL805, and 6.0 days at 25°C in 30 ppt for MS91-452.

Different isolates of *Streptococcus* sp. vary in their pathogenicity to Nile tilapia, but these variations are influenced by the temperature, and the salinity of the water in which they are held. Also, injury to the epithelium is a major contributing factor to the disease susceptibility under any environmental condition.

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REFERENCES

Al-Amoudi, M.M. 1987. Acclimation of commercially cultured *Oreochromis* species to seawater–an experimental study. Aquaculture 65:333-342.

Jo, Y. 1982. Streptococcal infections of cultured freshwater fishes. Fish Pathology 17:33-37.

Kitao, T., T. Aoki, and R. Sakoh. 1981. Epizootic caused by B-hemolytic *Streptococcus* species in cultured freshwater fish. Fish Pathology 15:301-307.

Miyazaki T., S.S. Kubota, N. Kaige, and T. Miyashita. 1984. A histopathological study of streptococcal disease in tilapia. Fish Pathology 19:167-172.

Moller, H. 1985. A critical review on the role of pollution as a cause of fish diseases. Pages 169-182 *in* A.E. Ellis, ed. Fish and Shell Fish Pathology. Academic Press, London.

Morgan, J.D., and G.K. Iwama. 1991. Effects of salinity on growth, metabolism and ion regulation in juvenile rainbow trout and steelhead trout, *Oncorhynchus mykiss* and fall chinook salmon, *Oncorhynchus tshawytscha.* Canadian Journal of Fisheries and Aquatic Sciences 48:2083-2094.

Ohnishi, K., and Y. Jo. 1986. Physiological characteristics and pathogenicity of B-hemolytic *Streptococcus* isolated from cultured ayu, *Plecoglossus altivelis.* Fish Pathology 21:9-13.

Plumb, J.A. 1991. Major diseases of striped bass and redfish. Veterinary and Human Toxicology 33:34-39.

Plumb, J.A., J.H. Schachte, J.L. Gaines, W. Peltier, and B. Carroll. 1974. *Streptococcus* sp. from marine fishes along the Alabama and northwest Florida coast of the Gulf of Mexico. Transactions of the American Fisheries Society 103:358-361.

Rasheed, V., and J.A. Plumb. 1984. Pathogenicity of a non-hemolytic Group B *Streptococcus* sp. in gulf killifish, *Fundulus grandis* Baird and Giard. Aquaculture 37:97-105.

Rodgers, L.J., and J.B. Burke. 1981. Seasonal variation in the prevalence of "red spot" disease in estuarine fish with particular reference to sea mullet, *Mugil cephalus* L. Journal of Fish Diseases 4:297-307.

Santos, J.I., A.O. Shigeoka, and H.R. Hill. 1982. Strain differences in virulence of Group B Streptococci. Pediatric Research 16:347-350.

SAS. 1982. SAS User's Guide: Basics. 1982 Edition, SAS Institute Inc., Cary, North Carolina.

Secombes, C.J., and T.C. Fletcher. 1992. The role of phagocytes in the protective mechanisms of fish. Annual Reviews of Fish Diseases 2:53-71.

Snieszko, S.F. 1974. The effects of environmental stress on outbreaks of infectious diseases of fishes. Journal of Fish Biology 6:197-208.

Suresh, A.V., and C.K. Lin. 1992. Tilapia culture in saline waters: a review. Aquaculture 106:201-226.

Tong, M.C., S.C. Chen, and S.S. Tsai. 1985. General septicemia of streptococcal infection in cage cultured tilapia, *Tilapia mossambica,* in southern Taiwan.

CAO Fisheries Series, No. 4. Fish Disease Research (VII) 95:192-200.
Walters, G.R., and J.A. Plumb. 1980. Environmental stress and bacterial infection in channel catfish, *Ictalurus punctatus,* Rafinesque. Journal of Fish Biology 17:177-185.
Wedemeyer, G., and J.W. Wood. 1974. Stress as a Factor in Fish Diseases. Fisheries Leaflet 38, U.S. Fish and Wildlife Service, Washington, D.C.