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Effects of temperature and salt concentration on latent *Edwardsiella ictaluri* infections in channel catfish

John A. Plumb, Craig Shoemaker

Southeastern Cooperative Fish Disease Project, Department of Fisheries and Allied Aquacultures and Alabama Agricultural Experiment Station, Auburn University, Alabama 36849, USA

ABSTRACT: Juvenile channel catfish *Ictalurus punctatus*, 10% of which had a natural, latent infection of *Edwardsiella ictaluri*, were held in water at 15 ± 2°C. Following elevation of the water temperature to 25°C for 28 d, 77% died with clinical *E. ictaluri* infections. This level of mortality was significantly higher ($p < 0.05$) than mortality attributable to *E. ictaluri* when the water temperature was raised to 18°C (10%) or to 30°C (23%). Channel catfish naturally infected with *E. ictaluri* were exposed to 0, 100, 1000, 2000, and 3000 mg l^{-1} NaCl at 25°C for 28 d. Mortalities of channel catfish held in NaCl concentrations of 0 and 100 mg l^{-1} (100% and 96% respectively) were significantly higher than those held in NaCl concentrations of 1000, 2000, and 3000 mg l^{-1} (33, 43, and 17%, respectively).

KEY WORDS: Channel catfish · Bacteria · Salt · Temperature · *Edwardsiella ictaluri*

INTRODUCTION

Edwardsiella ictaluri, the etiological agent of enteric septicemia of catfish (ESC), is one of the most serious bacterial diseases of farmed channel catfish *Ictalurus punctatus*. Since the first detection of *E. ictaluri* by Hawke (1979), and its subsequent description (Hawke et al. 1981), the pathogen has been found throughout much of the geographical area where channel catfish are cultured. *E. ictaluri* infections are most prevalent and most severe at water temperatures between 20 and 28°C (Francis-Floyd et al. 1987, Plumb 1988). However, recently ESC has occurred more frequently in the warm summer months and during the winter than previously reported. As epizootiological data accumulate on this pathogenic bacterium, it is being found in more diverse fish species and aquacultural environments. *E. ictaluri* has been confined to infections in freshwater fish species, however, an exception may be its presence in sea bass *Dicentrarchus labrax* (Blanch et al. 1990).

Some artesian well waters in western Alabama, USA, contain as much as 2500 mg l^{-1} of chloride (equivalent to about 4200 mg l^{-1} NaCl). Where these high salinity waters have been used in channel catfish culture ponds, *Edwardsiella ictaluri* infections seldom or never occur (G. Whitis, Fish Farming Center, Greensboro, AL, pers. comm.). As a result of these observations it has been proposed that large quantities of NaCl be added to freshwater channel catfish culture ponds as a method for preventing ESC where *E. ictaluri* is enzootic. Although NaCl has long been used therapeutically and prophylactically in cultured fish (Herwig 1979, Wellborn 1985), the effect of high salt concentrations on channel catfish infected with *E. ictaluri* has not been evaluated in the laboratory. The objectives of this study were to determine the effects of temperature and the effects of elevated salt concentration on a population of channel catfish with a naturally acquired, latent infection of *E. ictaluri*.

MATERIALS AND METHODS

Juvenile channel catfish (7 to 8 mo old), with a mean weight of 7.4 g and average total length of 10.5 cm, and

held in 15 ± 2°C water with <20 mg l^{-1} NaCl, were used in the experiments. Based on the occurrence of an occasional moribund individual showing minimal clinical signs of ESC, it was known that this particular lot of fish was latently infected with *Edwardsiella ictaluri*. The fish were treated with 167 mg l^{-1} formalin for 1 h to eliminate external parasites (*Epistylis* sp. and *Trichodina* sp.) and with a 1 h exposure to 20 mg l^{-1} of nitrofurazone on 3 consecutive days to eliminate a chronic *Flexibacter columnaris* infection 1 wk before the initial laboratory studies. To establish the background prevalence of *E. ictaluri* infection in these fish, 10 fish were killed with 100 mg l^{-1} of tricaine methanesulfonate (MS-222) and samples of their kidneys were streaked on *E. ictaluri* isolation medium (EIM) and incubated at 30°C (Shotts & Waltman 1990). Moribund and freshly dead fish were removed daily throughout the following 2 studies and similarly necropsied for bacterial infections. Isolated bacteria were identified on the basis of their biochemical characteristics (Shotts & Bullock 1975, Hawke et al. 1981, Austin & Austin 1987).

Experiments were carried out in static 57 l glass aquaria filled with 40 l of well water that had a total hardness and alkalinity of 40 mg l^{-1} of $CaCO_3$. Temperature was regulated with submersible aquarium heaters, and aeration was supplied with compressed air through air stones. Each aquarium was stocked with 10 fish in all replicates of all experiments. Each treatment was in triplicate with the exception of the temperature study involving fish held at 18°C; this treatment was in duplicate. At the end of each experiment the surviving fish were killed with MS-222, necropsied for bacterial infections, and buried.

To evaluate the effect of water temperature on the transition of *Edwardsiella ictaluri* from a latent stage to clinical infection, triplicate groups of channel catfish were held at 18 ± 1, 25 ± 1, and 30 ± 1°C for 28 d. Because water was static, 100 mg l^{-1} of NaCl was maintained to prevent methemoglobinemia (brown blood disease); however, nitrite levels were not measured (Tomasso et al. 1979). Fifty percent of the water was replaced at 5 d intervals and approximately 100 mg l^{-1} of NaCl was maintained.

The effect of NaCl on the transition of latent *Edwardsiella ictaluri* infection to a clinical infection was determined by exposing triplicate groups of fish to 0, 100, 1000, 2000, and 3000 mg l^{-1} of technical grade NaCl (American Chemical Society certified) on a continuous basis for 28 d. Latently *E. ictaluri* infected fish were moved from the 15°C holding tank to aquaria with freshwater, the appropriate quantity of NaCl was added to establish the desired concentration, and the water temperature was gradually increased to 25 ± 1°C. Approximately 50% of the water in each aquarium was replaced every 5 d and the desired salt concentration restored. At 24 h and 28 d after adding NaCl, water samples of the different treatments were analyzed for chloride ion concentration (Boyd 1979). Total mortality data from each treatment in each experiment were analyzed by 1-way analysis of variance using the general linear models procedure (GLM) to compare arcsine-transformed percent mortalities (SAS Institute 1985).

RESULTS AND DISCUSSION

A 10% *Edwardsiella ictaluri* carrier rate in the channel catfish population was recorded prior to the temperature and NaCl concentration experiments. Our data confirmed that *E. ictaluri* is most serious in cultured channel catfish when water temperatures are near 25°C, where a cumulative mortality of 77% due to *E. ictaluri* infection occurred over 28 d (Table 1). This mortality was significantly higher ($p < 0.05$) than *E. ictaluri* induced mortality of fish maintained at 18°C (10%) or in the fish held at 30°C (23%). However, cumulative total mortality attributable to all causes was 93% for fish held at 25°C, and 50% and 58%, respectively, for groups held at 18°C and 30°C; these mortalities were not significantly different from each other ($p > 0.05$) (Table 1). The lack of significance between total mortalities at the different temperatures may have been due to the presence of other bacterial etiologies (Table 2), or because there were only 2 replicates in the 18°C tests rather than 3, thus resulting in a Type II statistical error (Wise et al. 1993).

Only 1 of 24 bacterial isolates from fish in 25°C water was not *Edwardsiella ictaluri* (Table 2). However, only 2 of 9 bacterial isolates from the fish held at 18°C were *E. ictaluri* while 9 of the 18 isolates from the fish held at 30°C were *E. ictaluri*. These data further emphasize the importance of temperature in the epizootiology of

Table 1. *Ictalurus punctatus* infected with *Edwardsiella ictaluri*. Effects of temperature on mortality (values given as %) in latently infected channel catfish held at various temperatures for 28 d. Fish were held in 15°C water before the start of the experiments. Mortality values with different letters are significantly ($p < 0.05$) different from others in the same row

	18°C	25°C	30°C
No. fish stocked	20	30	30
Mortality			
Total	50[a]	93[a]	58[a]
Due to *E. ictaluri*	10[a]	77[b]	23[a]
Day of first *E. ictaluri* caused death	20	8	16

Table 2. *Ictalurus punctatus* infected with *Edwardsiella ictaluri*. Bacteria isolated from latently infected channel catfish held at 3 different temperatures and necropsied over 28 d. Fish were held in 15°C water before the start of the experiment. M-D: moribund or freshly dead fish necropsied during study period (some fish had dual infections); T: fish (survivors) necropsied at the end of the experiment (some fish had dual infections)

	18°C		25°C		30°C	
	M-D	T	M-D	T	M-D	T
No. of fish necropsied	10	10	28	2	17	13
Bacterial species isolated						
E. ictaluri	1	1	23	0	7	2
Aeromonas sp.	1	0	1	0	6	1
Pseudomonas sp.	0	0	0	0	0	1
Streptococcus sp.	5	0	0	0	0	1
Flexibacter columnaris	0	1	0	0	0	0
None	4	9	4	2	5	11

E. ictaluri infections. Other potentially pathogenic bacteria, such as the motile *Aeromonas* sp. group and *Streptococcus* sp., tended to occur at temperatures above and below, respectively, the optimum for clinical *E. ictaluri* infection. The time to first death due to *E. ictaluri* further supports the fact that 25°C is near the optimum temperature for ESC (Table 1). At 25°C the first *E. ictaluri* associated death occurred on Day 8 post temperature adjustment compared to Day 20 for fish held at 18°C and Day 16 for fish adjusted to 30°C. The only brown blood disease problem (methemoglobinemia) occurred at 30°C.

Although salt concentrations in our study were a fraction of that inhibiting growth of *Edwardsiella ictaluri* in culture media (15000 mg l^{-1}) (Plumb & Vinitnantharat 1989), increased NaCl concentrations had a positive effect on the reduction of total cumulative mortality of channel catfish with a latent *E. ictaluri* infection (Fig. 1). Mortality was significantly greater ($p < 0.05$) in the 0 and 100 mg l^{-1} treatment groups (100 and 96%, respectively) than in the 1000, 2000, and 3000 mg l^{-1} treatment groups (33, 43, and 17 %, respectively) (Table 3). Salt concentrations of 1000 and 3000 mg l^{-1} produced mortality patterns similar to each other and were not significantly ($p > 0.05$) different, but mortality in the 3000 mg l^{-1} treatment group was significantly lower ($p < 0.05$) than that in the 2000 mg l^{-1} treatment group. The higher average mortality in the 2000 mg l^{-1} treatment group was due to a high (70%) mortality in 1 of the 3 replicates compared to the 30% mortality that occurred in each of the other 2 replicates.

Bacteria other than *Edwardsiella ictaluri* were less of a problem in the 'temperature' study than in the 'salt' study (Table 4). Aquaria with a salt content of 1000 mg l^{-1} or higher had fewer fish deaths due to mixed infections of *E. ictaluri* and *Aeromonas* sp. than aquaria with 0 and 100 mg l^{-1} salt. No *Aeromonas* sp. were isolated from fish in 3000 mg l^{-1} salt. At termination of the study, 10% of the survivors in 1000 mg l^{-1} yielded *E. ictaluri* upon necropsy compared to 20% in the 2000 mg l^{-1} group and 0% in the 3000 mg l^{-1} group. This prevalence of *E. ictaluri* is very similar to the pre-experimental carrier level (10%) and indicates that the prevalence of this pathogen, as well as that for the motile *Aeromonas* sp., did not increase in the higher salinities. At present, it is not known if the low prevalence of bacterial infection in the fish held at 1000 to 3000 mg l^{-1} salt was because the bacteria were not transmitted to non-infected fish at these salt concentrations, or if the channel catfish became more resistant due to improved osmolarity. However, Plumb & Vinitnantharat (1989) showed that *E. ictaluri* grows in culture media with 10000 mg l^{-1} NaCl (equivalent to 6000 mg l^{-1} chloride), suggesting that the channel catfish benefit physiologically from the salt, thus increasing resistance.

Chloride analyses carried out 24 h after NaCl was added to the aquarium water indicated that initially

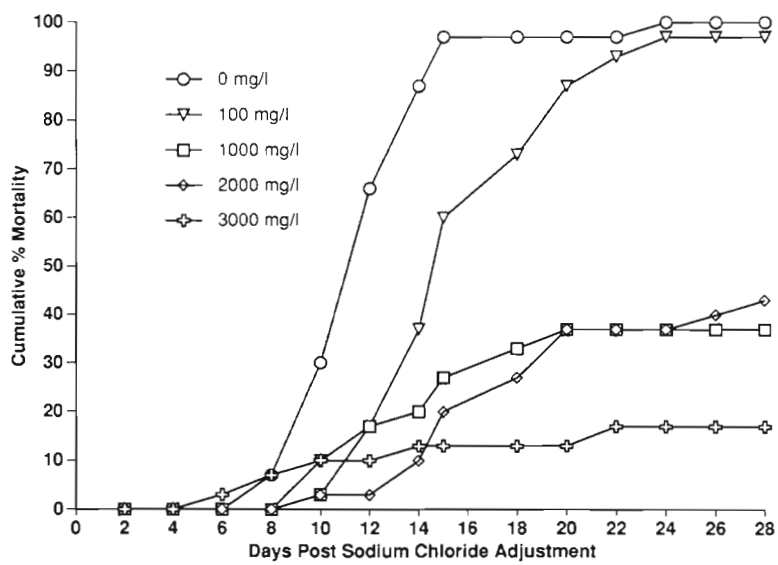

Fig. 1. *Ictalurus punctatus* infected with *Edwardsiella ictaluri*. Cumulative mortality of channel catfish naturally infected with latent bacteria and held in 0, 100, 1000, 2000, and 3000 mg l^{-1} of NaCl at 25°C for 28 d

Table 3. *Ictalurus punctatus* infected with *Edwardsiella ictaluri*. Effects of increased sodium chloride concentration on mortalities (values given as %) in latently infected channel catfish held at 25°C for 28 d. Mortality values with different letters are significantly different ($p < 0.05$) from others in the same row

	NaCl concentration (mg l^{-1}):				
	0	100	1000	2000	3000
No. of fish stocked	30	30	30	30	30
% mortality					
Total	100[a]	96[a]	33[bc]	43[b]	17[c]
Due to *E. ictaluri*	77[a]	93[a]	33[bc]	43[b]	17[c]
Day of first death due to *E. ictaluri*	7	9	10	9	6

Table 5. *Ictalurus punctatus* infected with *Edwardsiella ictaluri*. Chloride analysis of water to which sodium chloride had been added and in which infected catfish were held

NaCl added (mg l^{-1})	Chloride concentration (mg l^{-1})		
	Theoretical[a]	Measured[b]	
		24 h	28 d
0	0	–	18.3
100	60	66.1	73.1
1000	600	560.3	588.1
2000	1200	1155.0	1060.3
3000	1800	1700.0	877.5

[a] Based on 60 % (by weight) of NaCl being chloride ions
[b] Chloride ion concentration measured 24 h and 28 d after addition of NaCl (Boyd 1979)

chloride concentrations were near the desired level in all treatments (Table 5). At Day 28, the chloride concentrations were close to the desired level (Table 5) except in the 3000 mg l^{-1} replicates where, unfortunately, chlorides were only 877 mg l^{-1} compared to the desired concentration of near 1800 mg l^{-1}. This disparity could have been due to an error in replenishing the NaCl during a water exchange. However, in view of the effective disease control obtained with NaCl at the 1000 mg l^{-1} level (= 600 mg Cl^{-} l^{-1}), it may not be necessary to add NaCl to the 3000 mg l^{-1} level to inhibit *Edwardsiella ictaluri* infections.

The full benefit of adding salt to channel catfish ponds is not yet known but with few exceptions channel catfish culture is carried out in waters containing less than 200 to 400 mg l^{-1} of salinity (Boyd 1990). Although the salt content of static channel catfish ponds can be artificially increased, the initial cost of adding the required amounts per hectare would be high. However, once the salt is added to the water it should remain in the pond until it is drained. Furthermore, the cost of the salt would be an incentive for conserving treated water. Also, in areas where naturally high saline well water is available, NaCl supplements would not be as costly.

Suggested increased salinities are well within the range of channel catfish tolerance. Allen & Avault (1969) found that salinities of 10000 mg l^{-1} of total salinity or less permitted normal growth and survival of channel catfish. The 3000 mg l^{-1} NaCl concentration, theoretically 1800 mg l^{-1} chlorides, showed the lowest total mortality (17%) and no *Edwardsiella ictaluri* was isolated from fish 28 d after being introduced into that environment. The cost of sodium chloride treatment of ponds may be acceptable when the benefits of the treatment (increased survival of channel catfish) are considered. Mortalities of cultured channel catfish infected with *E. ictaluri* may reach 60 % or more if not treated and the use of salt is particularly appealing when the cost of medication is also taken into account. One of the problems with medication is the increased incidence of *E. ictaluri* resistance to oxytetracycline and ormethoprim-sulfamethoxine (Waltman & Shotts 1986, Plumb & Vinitnantharat 1990). Therefore, the addition of salt to channel catfish culture ponds may be a feasible management procedure for controlling ESC.

This laboratory study was initiated to evaluate management recommendations for non-saline waters that were stimulated by actual environmental conditions. Although the study leaves some important questions unanswered the trend of reduced losses due to *Edwardsiella ictaluri* as a direct response to elevated salinity is clear. Experiments are now being

Table 4. *Ictalurus punctatus*. Bacteria isolated from moribund and dead channel catfish exposed to 5 concentrations of sodium chloride at 25°C

	NaCl concentration (mg l^{-1}):				
	0	100	1000	2000	3000
No. of fish necropsied	30	29	10	13	5
No. of fish positive for:					
Edwardsiella ictaluri	24	28	10	13	5
Aeromonas sp.	6[a]	4[b]	1[b]	2[b]	0
No. of fish with no isolates	2[c]	1	0	0	0
No. of fish positive for *E. ictaluri* at termination[d]	0/0	0/1	1/10	2/10	0/10

[a] Two of six with dual infections of *Aeromonas* sp. and *E. ictaluri*
[b] Dual infection with *Aeromonas* sp. and *E. ictaluri*
[c] Brown blood disease suspected
[d] No. of fish positive for *E. ictaluri*/no. of fish necropsied at termination

designed to determine the mode of protection, lowest effective concentration, and the practicality of adding NaCl to channel catfish culture ponds to help prevent clinical ESC.

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