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Acclimation of Channel Catfish (*Ictalurus punctatus*) to Nitrite

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Nitrite (NO_2^-) induced methemoglobinemia has become a major water quality problem in commercial pond culture of channel catfish (Ictalurus punctatus). It is well established that as the molar ratio of $NO_2^-:Cl^-$ in the water decreases the % methemoglobin in fish also decreases (TOMASSO et al. 1979). Recently, we developed an empirical relationship between % methemoglobin in channel catfish and the $NO_2^-:Cl^-$ molar ratio in the pond water (SCHWEDLER & TUCKER in press). This relationship was developed from data obtained by sampling commercial catfish culture ponds in west central Mississippi. When we compared this relationship to results from aquarium studies, we consistently obtained lower % methemoglobin values in fish from pond environments when compared to those in the aquarium studies at the same $NO_2^-:Cl^-$ molar ratio. In the following study, we conducted three experiments to determine if channel catfish acclimate to nitrite. The ability to acclimate to nitrite may help explain the descrepancy between methemoglobin levels in fish from culture ponds and aquarium studies.

MATERIALS AND METHODS

Methods of Analysis. Chemical analyses of waters were conducted using standard methodology (AMERICAN PUBLIC HEALTH ASSOCIATION 1980): nitrite (diazotization), chloride (mercuric nitrate titration), pH (glass electrode), and total alkalinity (titration with standard acid). Percent methemoglobin was analyzed immediately (TIETZ 1970) on blood drawn from the caudal vessel into heparinized evacuated tubes.

Experiment 1. Channel catfish fingerlings (45 g average weight) were obtained from a flow-through holding tank at the Catfish Research Unit, Delta Branch Experiment Station, Stoneville, MS. These fish had never been exposed to > 0.01 mg/L NO_2^--N (NO_2^-/Cl^- molar ratio < 0.003). These fish will be designated "unacclimated". Fifty fish were placed in an 0.6-m^3 holding cage stationed in an 0.06-ha earthen pond. This pond held approximately 2000 fingerling channel catfish (0.55 g average weight) that had been exposed to > 3.0 mg/L NO_2^--N (NO_2^-/Cl^- molar ratio approximately 0.26) for at least the preceding two weeks. These fish will be designated "acclimated". The pond was constantly aerated and dissolved oxygen concentrations were never < 5 mg/L. Mean afternoon water temperatures were 27 ± 2°C during the study. The total alkalinity of the pond water was 277 mg/L as $CaCO_3$, typical of west central Mississippi catfish culture ponds.

Immediately prior to and at 1, 2, 4, 7, 9, 15, 20 and 28 days after stocking "unacclimated" fish, 6 "acclimated" and 6 "unacclimated" fish were randomly removed for determination of % methemoglobin. Water samples were also collected on each sampling date and analyzed for NO_2^--N and Cl^-.

Experiment 2. Twelve "unacclimated" channel catfish (30 g average weight) were obtained from the holding tank as above. Six of these fish were stocked into each of two 80-L aquaria. Twelve "acclimated" fish (35 g average weight) were obtained from a pond at the Catfish Research Unit. These fish had been exposed to > 3.0 mg/L NO_2^--N (NO_2^-/Cl^- molar ratio approximately 0.9) for at least two weeks. Six of these fish were stocked into another two 80-L aquaria. Tests were run in 25 ± 2°C, O_2 saturated water with a total alkalinity of 225 mg/L as $CaCO_3$ and 20 mg/L Cl^-. The pH remained at 8.0 ± 0.2 throughout the test. Immediately after stocking the fish, 4.72 mg/L NO_2^--N (as $NaNO_2$) was added to each aquarium. This established a NO_2^-/Cl^- molar ratio of 0.60. Fish were removed 24 h later for determination of % methemoglobin. Water samples were also analyzed to verify NO_2^--N and Cl^- concentrations.

Experiment 3. "Unacclimated" channel catfish (30 g average weight) were obtained from the holding tank as above. Six fish were stocked into each of six 80-L aquaria and held 48 h before testing. Temperature and water quality were as above. In three aquaria, 3.28 mg/L NO_2^--N was added (as $NaNO_2$) in a single dose to establish a NO_2^-/Cl^- molar ratio of 0.42. Fish were collected and percent methemoglobin determined 24 h later. In the other three aquaria, nitrite was added in five equal daily doses to total 3.28 mg/L NO_2^--N. Fish were collected 24 h after the last dose for methemoglobin analysis. Water samples were also analyzed for NO_2^--N and Cl^- at the time of fish collection.

RESULTS

Experiment 1. In "acclimated" fish , % methemoglobin decreased during the study period as the $NO_2^-:Cl^-$ molar ratio decreased (Table 1). The mean % methemoglobin in these fish was comparable to the value predicted by the equation developed by SCHWEDLER & TUCKER (in press). With the exception of two dates, the predicted value lies within one standard error of the mean of measured values. Initially, "unacclimated" fish had 6 ± 1 % methemoglobin (Table 1). This is in the range of nominal values found by TOMASSO et al. (1979) for channel catfish exposed to very low $NO_2^-:Cl^-$ molar ratios. Within one day after being stocked into the pond, methemoglobin had increased to 56 ± 5%. The highest % methemoglobin value recorded occurred on the second day after stocking. For at least four days, the "unacclimated" fish had % methomoglobin values more than twice as high as "acclimated" fish. Values for % methemoglobin for "unacclimated" fish did not decrease to levels comparable to those for "acclimated" fish until fifteen days after stocking.

Table 1. Percent methemoglobin (mean ± SE) for fish in a 0.06 ha pond ("acclimated") and fish previously unexposed to nitrite ("unacclimated") that were stocked into the pond. Predicted methemoglobin values were derived using the equation of SCHWEDLER & TUCKER (in press).

Time After Stocking (days)	NO_2^--N (mg/L)	Cl^- (mg/L)	NO_2^-/Cl^- molar ratio	Methemoglobin Unacclimated (%)	Methemoglobin Acclimated (%)	Methemoglobin Predicted (%)
0*	<0.01	8	<0.003	6 ± 1		7
0	3.00	29	0.26		32 ± 4	28
1	2.94	31	0.24	56 ± 5	24 ± 4	26
2	2.88	31	0.24	62 ± 2	24 ± 5	25
4	2.61	32	0.21	46 ± 4	17 ± 4	23
7	2.79	34	0.20	32 ± 3	19 ± 3	23
9	2.60	35	0.19	31 ± 4	19 ± 4	22
15	2.26	33	0.17	29 ± 3	21 ± 4	21
20	2.06	31	0.17	20 ± 3	18 ± 4	20
28	2.07	32	0.16	21 ± 2	18 ± 5	20

* Water quality in the holding tank from which the unacclimated fish were obtained.

Experiment 2. Prior to stocking into the aquaria, both "acclimated" and "unacclimated" fish had % methemoglobin levels comparable to the predicted value for the $NO_2^-:Cl^-$ molar ratio in the water from which they were obtained (Table 2). After 24 h, methemoglobin in "acclimated" fish decreased from 81 ± 3% to 60 ± 3 % in response to the lower $NO_2^-:Cl^-$ molar ratio in the aquarium water. The value of 60% methemoglobin is slightly higher than the predicted value of 55%. "Unacclimated" fish developed 82 ± 3% methemoglobin. This is considerably higher than both the predicted value (55%) and the value obtained for "acclimated" fish (60 ± 3%). Analysis of variance indicated a highly significant difference ($P < 0.01$) between the final mean % methemoglobin in "acclimated" and "unacclimated" fish exposed to the same $NO_2^-:Cl^-$ molar ratio in the aquaria.

Experiment 3. The mean methemoglobin in fish receiving a single dose of 3.28 mg/L NO_2^--N was 71 ± 2% (Table 3). This is much higher than the predicted value of 39% methemoglobin. The final NO_2^--N concentration in aquarium water dosed daily with 0.65 mg/L NO_2^--N was 3.43 mg/L. This concentration was slightly higher than in aquaria receiving one dose, presumably the result of cumulative errors in weighing the $NaNO_2$. The fish from aquaria dosed daily had a final mean % methemoglobin of 50 ± 2%; higher than the predicted value of 41% but significantly ($P < 0.01$) lower than the 71% value for fish receiving a single dose of nitrite even though the final NO_2^-/Cl^- molar ratio was approximately 0.42 in all aquaria.

Table 2. Percent methemoglobin (mean ± SE) for two groups of fish before and after 24 h exposure to approximately 4.7 mg/L NO_2^--N and 20 mg/L chloride (NO_2^-/Cl^- molar ratio 0.60). One group ("acclimated") was from a pond with a high $NO_2^-:Cl^-$ molar ratio (0.9) and the other group ("unacclimated") from a tank with a low molar ratio (<0.003). Predicted methemoglobin values were derived from the equation of SCHWEDLER & TUCKER (in press).

	Pretreatment				Posttreatment			
	NO_2^--N (mg/L)	Cl^- (mg/L)	methemoglobin measured (%)	methemoglobin predicted (%)	NO_2^--N (mg/L)	Cl^- (mg/L)	methemoglobin measured (%)	methemoglobin predicted (%)
Acclimated	3.18	9	81 ± 3	78	4.73	20	60 ± 3	55
Unacclimated	<0.01	8	8 ± 2	7	4.70	20	82 ± 3	54

Table 3. Percent methemoglobin (mean ± SE) in fish in aquaria receiving a single dose of 3.28 mg/L NO_2^--N and fish in aquaria receiving five equal daily doses of nitrite. The five daily doses were each approximately 0.68 mg/L NO_2^--N. Predicted methemoglobin values were derived using the equation of SCHWEDLER & TUCKER (in press).

	Final concentrations		Methemoglobin	
	NO_2^--N (mg/L)	Cl^- (mg/L)	measured (%)	predicted (%)
Single Dose	3.28	20	71 ± 2	39
Daily Doses	3.43	20	50 ± 2	41

DISCUSSION

The results of Experiments 1 and 2 strongly suggest that channel catfish acclimate to nitrite. In both tests fish that had not previously been exposed to > 0.01 mg/L NO_2^--N (NO_2^-/Cl^- molar ratio < 0.003) developed higher levels of methemoglobin than fish with an immediate past history of exposure to relatively high NO_2^-/Cl^- molar ratios. This apparently explains the failure of the relationship developed by SCHWEDLER & TUCKER (in press) to adequately predict % methemoglobin in fish in our previous aquarium studies. The fish we had previously used in aquarium studies were obtained from environments with very low nitrite concentrations and because of the short term nature of static bioassays (24 - 96 h), the fish did not acclimate to test nitrite levels. In the present study (Experiment 2), fish obtained from a pond with a relatively high NO_2^-/Cl^- molar ratio and subjected to bioassay conditions developed methemoglobin levels comparable to that predicted for the NO_2^-/Cl^- molar ratio in the aquarium water.

The relationship found by SCHWEDLER & TUCKER (in press) was developed from data obtained from commercial culture ponds. The actual source of nitrite in these ponds is unknown. Nitrite is an intermediate in both denitrification and nitrification and both biochemical pathways have been proposed as the source of nitrite in catfish ponds (HOLLERMAN & BOYD 1980). Whatever the source, nitrite concentrations in culture ponds increase gradually over a period of time. Maximum nitrite concentrations are usually measured from several days to two weeks after the onset of the episode of increasing nitrite concentrations. During this time, the fish present in the pond are exposed to continually increasing nitrite levels and apparently partially acclimate to the conditions. We attempted to reproduce these conditions in Experiment 3. The fish exposed to increasing $NO_2^-:Cl^-$ molar ratios developed lower % methemoglobin levels than fish in aquaria where the NO_2^-/Cl^- molar ratio increased suddenly although the final NO_2^-/Cl^- molar ratios were essentially equal.

The results of this study may have implications to both research and commercial culture pond management. Virtually all research dealing with nitrite induced methemoglobinemia in fish has been performed using standard toxicity testing protocol. Although the data obtained may be comparable from one laboratory to another, the results may overestimate the levels of methemoglobin developed by fish in commercial culture situations at the same $NO_2^-:Cl^-$ molar ratio. Fortunately, management decisions and practices based on these results will err on the conservative side and allow for a margin of safety.

In west central Mississippi, nitrite induced methemoglobinemia is currently managed by adding a chloride salt to decrease the $NO_2^-:Cl^-$ molar ratio to 0.25 ($NO_2^-:Cl^-$ = 0.33 by weight). This results in methemoglobin levels between 25 and 30%. This treatment has been found sufficient to prevent the death of the fish present in the pond. However, if the pond is stocked with fish previously unexposed to high NO_2^-/Cl^- molar ratios, the stocked fish may develop methemoglobin levels much higher than the indigenous population as demonstrated by Experiment 1. The resulting severe methemoglobinemia in the stocked fish may result in death or stress, particularly if dissolved oxygen concentrations are marginally low. Commercial pond managers should be aware of this potential and base management decisions accordingly.

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