Bull. Environ. Contam. Toxicol. (1985) 34:754–760

Bulletin of Environmental Contamination and Toxicology

Swimming Performance of Channel Catfish (*Ictalurus punctatus*) after Nitrite Exposure

Donald E. Watenpaugh and Thomas L. Beitinger*

Department of Biological Sciences and Institute of Applied Sciences, North Texas State University, Denton, TX 76203

Nitrite can accumulate in natural aquatic or aquacultural systems when nitrification is disrupted (Sawyer 1960). Such circumstances can result in concentrations of nitrite toxic to fish. Salmonids and channel catfish, both commonly cultured commercially, are especially susceptible to nitrite toxicity (Russo et al. 1974; Huey et al. 1980). Toxic effects of nitrite include oxidation of hemoglobin to methemoglobin, a form incapable of binding oxygen (Tomasso et al. 1979). Tissue hypoxia produced by methemoglobinemia has been identified as a possible cause of death in nitrite-exposed catfish (Huey et al. 1980).

Several investigators have identified swimming performance as a potentially sensitive indicator of sublethal stress in fish (Sprague 1971; Waldichuk 1979; Wedemeyer and McLeay 1981; Schneider and Connors 1982). Farlinger and Beamish (1977) subdivided swimming performance of fish into three general categories: sustained swimming that utilizes only aerobic metabolism and can continue indefinitely, prolonged swimming that depends on both aerobic and anaerobic metabolism and cannot be sustained indefinitely, and burst swimming that lasts only seconds and thus is supported by anaerobic metabolism. Tests of prolonged swimming are generally considered most useful in sublethal stress assessment, as they draw on both major biochemical energy sources (Brett 1964). No research to date has quantified either the swimming performance of I. punctatus or effects of nitrite exposure on swimming of any species. Our purpose was to determine if nitrite exposure affects the prolonged swimming performance of channel catfish, and to delineate the extent that methemoglobinemia resulting from nitrite exposure correlates with performance.

MATERIALS AND METHODS

Channel catfish (Ictalurus punctatus, fingerlings, wet wt. = 12.6 + 2.6 g, S.L. = 105 + 8 mm, ($\bar{X}$ + s) obtained from a local fish hatchery were acclimated to and held at 30 + 0.5°C ($\bar{X}$ + s) for a minimum of 4 weeks. Channel catfish were fed catfish chow daily

*Correspondence and reprint requests

until 2 days before experimentation. Two thermoregulators controlled temperature in a 450 L "living stream" (Frigid Units, Inc.) that continuously circulated holding water through charcoal filters. Aerated E.P.A. "hard" water reconstituted from deionized water was used for acclimation and holding (U.S. EPA 1975). Since chloride has been shown to inhibit nitrite toxicity in channel catfish in addition to reducing disease, 5 g/L Cl^-, as NaCl, was also added (Tomasso et al. 1979). Nitrite-N levels did not exceed 0.1 mg NO_2-N/L during holding.

Channel catfish were exposed to nitrite for 24 h in all glass aquaria containing 30 L of aerated, reconstituted, hard water. Aquaria were partially immersed in a water bath to maintain temperature at 30 $\pm$ 0.1°C ($\bar{X}$ $\pm$ s). Nominal exposure concentrations of 0.0, 0.5, 1.0, and 1.5 mg NO_2-N/L were chosen to produce a graded yet sublethal increase in methemoglobin concentration of channel catfish blood (Huey et al. 1980). Nitrite concentration was measured with the azo-dye method (A.P.H.A. 1975). Nitrite concentrations were maintained to $\pm$ 0.05 mg NO_2-N/L throughout the 24 h exposure period by addition of reagent grade sodium nitrite or deionized water, as necessary. Five fish with visibly similar lengths were withdrawn from holding for each exposure, and each concentration was replicated.

Swimming performance of channel catfish was evaluated as time (in min) to exhaustion in a recirculating system designed to produce identical current flow through five adjacent channels. The channels, constructed of plexiglas, were 75 cm long, 6.5 cm wide, and had laterally curved bottoms. Plastic mesh baffles moderated water flow in the channels. During swimming trials, individual fish were segregated in the downstream 30 cm section of each channel by a plastic mesh baffle at the front and an electrified grid at the downstream end. The grid was charged during testing with 6V D.C. from a Grass S9 stimulator. A 1.5 horsepower 220V A.C. centrifugal pump moved water from a 170 L reservoir tank to the swim channels, which drained back into the tank. Water flow into channels was controlled with valves in the pipes at the head of each channel. A single movable plexiglas vane behind the channels controlled back pressure and thus outflow. Water depth in channels was maintained at 6 cm. Current velocity was measured in cm/sec before and after swimming tests by counting RPM's of a 50 cm circumference paddle wheel.

Upon completion of a particular 24 h exposure, the five fish from that exposure were introduced one each to the swimming channels. Initially water flow was 12.5 $\pm$ 0.5 cm/sec ($\bar{X}$ $\pm$ s), or equivalent to about 1.2 body lengths per second. Five minutes were allowed for fish to become familiar with their channels and to orient to the current. During the next 5 min, current was increased at 1 min intervals to a maximum velocity of 37.3 $\pm$ 2.5 cm/sec, or about 3.5 body lengths per second, in all channels. When a fish no longer avoided the electrified grid at the downstream end of its channel, swim time (from introduction) was recorded and the fish was removed. Blood collected from the severed caudal peduncle was

then analyzed for hemoglobin and methemoglobin with methods of Hainline (1958) and Evelyn and Malloy (1938), respectively. Dissolved oxygen (± 0.05 mg O_2/l) and temperature (± 1°C) were measured in test water before and after each trial with a D.O. meter (Rexnord Instrument Co.).

The relationships between nitrite exposure, percent methemoglobin (% MHb), and time of channel catfish were described with least squares linear regression. Because Shapiro and Wilk's W statistic indicated data were normally distributed (α=0.01; Zar 1974) and Bartlett's test found sufficient homoscedasticity (α=0.001; S.A.S. 1982), for parametric testing differences in mean swim time and % MHb of the various exposure groups were also analyzed by analysis of variance (ANOVA) and Duncan's multiple range test (Zar 1974). All analyses were conducted with Statistical Analysis System procedures (S.A.S. 1982).

RESULTS AND DISCUSSION

Fish usually encountered the electrified grid at the downstream end of their channels soon after they were placed in the apparatus, and avoided it thereafter until they approached exhaustion. Tail beat frequency was usually constant, yet many test fish occasionally used "burst and coast" swimming, especially as they neared the test endpoint. A single fish from the 1.5 mg NO_2-N/L exposure was intolerant of any increase in current velocity above the initial 12.5 cm/sec, and no catfish from either of the highest exposures swam against the maximum rate of 37.3 cm/sec for more than 1 min (11 minutes after introduction). Although four unexposed catfish exhibited a similar inability to swim against currents exceeding three body lengths/sec, four other control fish swam for more than 1 h after introduction. The longest post-introduction swim time recorded was 77 min, and the control mean was 40 ± 30 min ($\bar{X}$ ± s). Channel catfish from the 0.5 mg NO_2-N/L exposure had swim times intermediate between controls and higher exposures. No fish died before its blood was collected. Water temperature during swimming tests averaged 28 ± 1°C, and dissolved oxygen ranged from 7.5 to 7.9 mg O_2/L.

The percentage of hemoglobin converted to methemoglobin (%MHb) was significantly correlated (r^2=0.64, $p<0.001$) to nitrite concentration, as expressed in the following equation: %MHb = 22.0 + 42.9 (mg NO_2-N/L). Mean %MHb levels in the three nitrite exposure groups ranged from 54 to 80%, and were significantly higher than controls (ANOVA F = 32.85, $p<0.001$). Furthermore, Duncan's multiple range test distinguished between %MHb means of catfish from the lowest and highest exposures (Table 1).

Exposure of channel catfish to nitrite significantly reduced their prolonged swimming performance, as best described by a linear regression of the base 10 logarithm of swim time (in minutes) on nitrite-N exposure concentration: Log_{10} (swim time) = 1.42 - 0.38 (mg NO -N/L); $p<0.001$, r^2=0.42, n=40 (Figure 1).

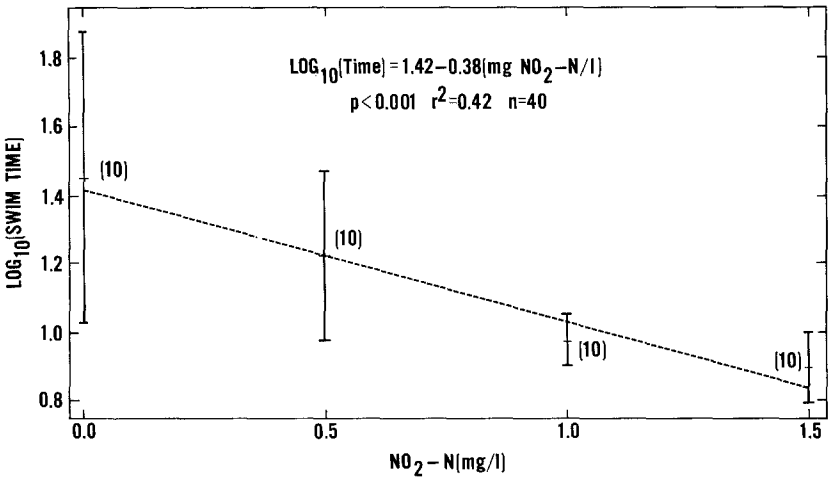

Figure 1. The base ten logarithm of channel catfish swim time (in minutes) plotted against 24 h nitrite exposure. Means, ± standard deviation, and sample size (parenthesis) are given for each group.

Table 1. Percent methemoglobin (%MHb) and swim time of channel catfish exposed for 24h to different concentrations of nitrite. Standard deviations are in parenthesis. Vertical lines next to percent methemoglobin and swim times are Duncan's multiple range test groups (α=0.05).

$mgNO_2$-N/L	n	%MHb	swim time (min)
0.0	10	13.4 (7.2) |	40 (30) |
0.5	10	54.0 (23.7) |	20 (15) |
1.0	10	69.7 (15.0) | |	9 (1) |
1.5	10	80.2 (15.6) |	8 (2) |

Analysis of variance on untransformed mean swim times indicated highly significant differences between means ($F = 7.58$, $p<0.001$), yet only the control mean was significantly different according to Duncan's test (Table 1).

Catfish swim time performance was inversely correlated with percent methemoglobin ($p<0.001$, $r^2=0.25$, $n=40$). Notable departures from the best-fit linear model include a fish from the low exposure which swam for 57 min with 76.8% MHb, and control fish that swam for less than 12 min, yet had MHb levels below 10%. No significant correlations were found between swim time and total

hemoglobin, standard length, weight, condition factor, or swim channel (α=0.05), probably because test fish were selected to be similar in size.

Although standardization of a methodology to evaluate fish swimming performance is desirable for comparative purposes, variations in fish size and hypotheses under test make acceptance of a standardized testing format unlikely. Most investigations of fish swimming performance have dealt with effects of major abiotic factors (e.g., oxygen, temperature) during forced activity, yet others have attempted to quantify sublethal stress in fish exposed to toxic substances. Lemke and Mount (1963) found that concentrations of ABS detergent damaging to gills did not affect bluegill swimming ability. In contrast, Macleod and Smith (1966) showed that sublethal levels of pulpwood fiber reduce fathead minnow swimming performance. Critical swim speed of rainbow trout was decreased by prior exposure to copper or low pH (Waiwood and Beamish 1978). Prolonged swimming performance of fish has been determined most often as critical swim speed; however, fatigue time at a set speed and other methods have also been employed (Wedemeyer and McLeay 1981).

The prolonged swimming ability of unexposed channel catfish in this study was variable and slightly lower than that recorded for other similar sized non-scombroid fish (Blake 1983). A relatively low swimming stamina is understandable in terms of the inactive existence of channel catfish in lentic depths (Miller and Robison 1973) and body shape of this species. Although mean percent methemoglobin of control catfish was high in this investigation, the overall relationship between methemoglobin formation and acute nitrite exposure observed here is similar to that reported by others (Tomasso et al. 1979; Huey et al. 1980; Huey et al. 1984).

The reduction in prolonged swimming performance of nitrite-exposed channel catfish is probably due in part to methemoglobin formation. Methemoglobin in blood reduces delivery of oxygen to tissues by making hemoglobin unavailable for oxygen transport and by shifting the oxygen dissociation curve to the left (Bodansky 1951). Such a shift increases the affinity of hemoglobin for oxygen, thereby making it less able to unload oxygen to the tissues. Channel catfish with larger percentages of their hemoglobin oxidized to methemoglobin by nitrite must rely more heavily on anaerobic glycolysis than aerobic metabolism to fuel locomotor activity. Glycolysis, in addition to being only a short term energy source for vertebrates, produces lactate as an end product. Muscle glycolysis still eventually requires oxygen to metabolize the toxic lactate after it is transported to the liver (Bartholomew 1982). Reduced aerobic metabolism of channel catfish with methemoglobinemia should limit prolonged swimming performance. The statistically significant but low correlation coefficient of the equation relating swim time to % MHb, however, implies that other factors are also influencing catfish swimming performance. Intraspecific variation in aerobic and anaerobic capacity may be among those factors: both Caillouet (1968) and

Scott and Rogers (1981) have noted high variability of blood glucose and lactate of channel catfish after exposure to hypoxia.

In conclusion, prolonged swimming performance as measured by swim time to exhaustion proved to be a sensitive yet imprecise indicator of sublethal nitrite toxicity in channel catfish. In spite of the variable performance of fingerlings swimming 3.5 standard body lengths per second, the potential for relatively low nitrite concentrations to reduce scope for activity of channel catfish is apparent.

Acknowledgements. We would like to thank Hal Ferguson, Steve Timmons, Chris Moses, and Stan Todd for their technical assistance, Donna Argo and Shannon Weaver for their preparation of the manuscript, and an anonymous reviewer for his/her comments. A North Texas State University Faculty Research Grant to T.L. Beitinger provided funds for this research.

REFERENCES

American Public Health Association (1975) Standard Methods for the Examination of Water and Wastewater. 14th Ed. Washington: APHA, pp 380-383

Bartholemew GA (1982) Energy Metabolism. In: Animal Physiology: Principles and Adaptations, 4th Ed. MS Gordon, Ed. Macmillan Publishing Co, New York, NY, p 46-83

Blake RW (1983) Fish Locomotion. Cambridge University Press, Cambridge, United Kingdom

Bodansky O (1951) Methemoglobinemia and methemoglobin-producing compounds. Pharmacol Rev 3:144-196

Brett JR (1964) The respiratory metabolism and swimming performance of young sockeye salmon. J Fish Res Board Can 21:1183-1226

Caillouet CW (1968) Lactic acidosis in channel catfish. J Fish Res Board Can 25:15-23

Evelyn HA, Malloy HT (1938) Microdetermination of oxyhemoglobin, methemoglobin, and sulfhemoglobin in a single sample of blood. J Biol Chem 126:655-662

Farlinger S, Beamish FWH (1977) Effects of time and velocity increments on the critical swimming speed of largemouth bass (Micropterus salmoides). Trans Am Fish Soc 106:436-439

Hainline A (1958) Hemoglobin. In: Standard Methods of Clinical Chemistry. D Seligson, Ed. WB Saunders, Philadelphia, Pennsylvania p 268-269

Huey DW, Beitinger TL, Wooten MC (1984) Nitrite-induced methemoglobin formation and recovery in channel catfish (Ictalurus punctatus) at three acclimation temperatures. Bull Environ Contam Toxicol 32:674-681

Huey DW, Simco BA, Criswell DW (1980) Nitrite-induced methemoglobin formation in channel catfish. Trans Am Fish Soc 109:558-562

Lemke AE, Mount DI (1963) Some effects of alkyl benzene sulfonate on the bluegill, Lepomis macrochirus. Trans Am Fish Soc 92:372-378

Macleod JC, Smith LL, Jr. (1966) Effect of pulpwood fiber on oxygen consumption and swimming endurance of the fathead minnow, Pimephales promelas. Trans Am Fish Soc 95:71-84
Miller RJ, Robison HW (1973) The Fishes of Oklahoma. Oklahoma State University Press, Stillwater, Oklahoma
Russo RC, Smith CE, Thurston RV (1974) Acute toxicity of nitrite to rainbow trout (Salmo gairdneri). J Fish Res Board Can 31:1653-1655
S.A.S. Institute Inc. S.A.S. User's Guide: Statistics. 1982 Edition. S.A.S. Institute, Inc, Cary, North Carolina
Sawyer C (1960) Chemistry for Sanitary Engineers. McGraw Hill, New York, New York
Schneider MJ, Connors TJ (1982) Effects of elevated water temperature on the critical swim speeds of yearling rainbow trout, Salmo gairdneri. J Thermal Biol 7:227-229
Scott AL, Rogers WA (1981) Haematological effects of prolonged sublethal hypoxia on channel catfish Ictalurus punctatus (Rafinesque). J Fish Biol 18:591-601
Sprague JB (1971) Measurement of pollutant toxicity to fish - III:sublethal effects and "safe" concentrations. Water Res 5:245-266
Tomasso JR, Simco BA, Davis KB (1979) Chloride inhibition of nitrite-induced methemoglobinemia in channel catfish (Ictalurus punctatus). J Fish Res Board Can 36:1141-1144
Waiwood KG, Beamish FWH (1978) Effects of copper, pH, and hardness on the critical swimming performance of rainbow trout (Salmo gairdneri Richardson). Water Res 12:611-619
Waldichuk M (1979) Review of the problems. Phil Trans R Soc Lond 286:399-424
Wedemeyer GA, McLeay DJ (1981) II. Methods for determining the tolerance of fishes to environmental stressors. In: Stress and Fish. AD Pickering, Ed. Academic Press, New York, New York, p 247-275
Zar JH (1974) Biostatistical Analysis. Prentice-Hall Inc, Englewood Cliffs, New Jersey

Received June 21, 1984; Accepted July 26, 1984.