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Short Communication

FRESHWATER MUSSEL DIE-OFF ATTRIBUTED TO ANTICHOLINESTERASE POISONING

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Abstract—In 1990, we investigated a die-off of freshwater mussels in north-central North Carolina. An estimated 1,000 mussels of several species were found dead or moribund, including about 111 Tar spinymussels (*Elliptio steinstansana*), a federally listed endangered species. The die-off occurred during a period of low flow and high water temperature in a stream reach dominated by forestry and agriculture. Pathological examinations did not show any abnormalities and indicated that the die-off was an acute event. Chemical analyses of mussels, sediments, and water revealed no organophosphorus or carbamate pesticides. Cholinesterase activity in adductor muscle from Eastern elliptios (*Elliptio complanata*) collected at the kill site and downstream was depressed 73 and 65%, respectively, compared with upstream reference samples. The depression is consistent with a diagnosis of anticholinesterase poisoning. This is the first documented case in which cholinesterase-inhibiting compounds have been implicated in a die-off of freshwater mussels.

Keywords—Freshwater mussels Poisoning Cholinesterase

INTRODUCTION

Of the approximately 300 species and subspecies of freshwater mussels in North America, nearly one-half have become extinct or endangered or have populations that have declined to the point that federal protection may be necessary [1]. National trends are mirrored in North Carolina, a state with about 70 species of freshwater mussels, including 4 species that are federally listed as endangered and 13 species that are candidates for federal listing.

Early work indicated that siltation, pollution, commercial harvest, and dams were important issues in the decline of freshwater mussel fauna in the eastern half of the United States [2–4]. No doubt, some of these factors acted insidiously, altering sediment and water-quality characteristics sufficiently to result in gradual reductions in recruitment and survival of vulnerable species. Mussel die-offs, episodic events that typically involve hundreds to thousands of mussels dying during a short period (days to weeks), have increased in frequency since 1982 [5]. Neves [5] concluded that mussel die-offs since the early 1980s exceed the normal occurrence of isolated incidents that are part of the natural population dynamics of this fauna. The etiology of these die-offs remains largely unknown. For example, of 16 die-offs reported by Neves [5], in only two could possible causative factors be identified.

We report on a die-off of freshwater mussels in a stream that supports the largest of three remaining populations of Tar spinymussel (*Elliptio steinstansana*), a species on the federal endangered list, as well as populations of three additional species that are proposed for federal listing [6,7].

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DIE-OFF INVESTIGATION

Die-off description

At 1030 h on August 3, 1990, biologists conducting a mussel survey of Swift Creek, Nash County, North Carolina, discovered a die-off of freshwater mussels in progress. Dead mussels were observed along a 7-km reach of the creek and involved at least 5 ha of surface area; the downstream extent of the die-off was never definitively identified (Fig. 1). The Eastern elliptio was one of the more common species in the stream and composed the largest portion of the die-off. Among the estimated 1,000 dead and moribund mussels of several species, 111 Tar spinymussels were counted. The fate of the moribund mussels was not followed. No dead fish or other animals were reported at the site.

At 1730 h on the day the die-off was first noticed, the dissolved oxygen content of creek water at the site was 8.4 mg/L, the pH was 6.64, and the water temperature was 23°C. Water temperature was 25°C and dissolved oxygen content was 6.0 mg/L at ~1000 h on July 31 at Hilliardston, North Carolina, the closest ambient monitoring station on Swift Creek, about 10 km upstream. Water flow at the Hilliardston station on August 3 was only about 19% of the average daily flow (161 cfs) because of drought conditions that existed during summer 1990 in eastern and central North Carolina. The 31-cfs flow on August 4 was the lowest recorded during the period October 1, 1989, to September 30, 1990 [8]. The 6.0 mg dissolved oxygen/L on July 31, 1990, was the lowest recorded during the same period.

The die-off occurred in a stream reach dominated by forestry and agriculture, with few industrial or urban discharges. The principal crops in the area were sweet potatoes, cotton, tobacco, corn, and soybeans. Lannate (a.i., methomyl) and

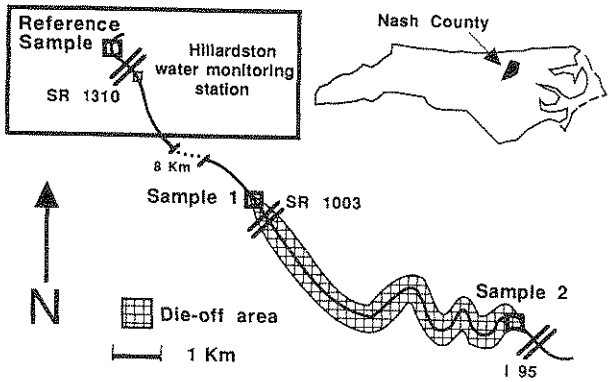

Fig. 1. Location of freshwater mussel die-off and collection sites on Swift Creek, Nash County, North Carolina, 1990.

Orthene (a.i., acephate) were the two most commonly used insecticides in Nash County during 1990, although other organophosphorus and carbamate pesticides, as well as pyrethroid insecticides, were used on croplands in the watershed. The county extension agent reported little use of agricultural chemicals in the vicinity during August.

There were eight permitted point-source discharges into Swift Creek, but only two upstream of the die-off site. The two permitted discharges upstream of the die-off site were for treated domestic wastewater, with discharge volumes of 10,600 and 15,140 L/d. These discharges equated to 0.01% of average daily flow and 0.2% of the annual minimum 7-d consecutive low flow that on average will be exceeded in 9 out of 10 years [9].

Potential sources of toxic substances included an agricultural irrigation pumping station about 1 km upstream from the die-off site. A researcher on site 8 d prior to the die-off reported that the irrigation pump station was active and that pesticide containers were present at the pump site.

Moribund and dead mussels were collected from the die-off site for chemical analyses and other testing. At the time of collection, dead mussels were not decomposed and considered to be freshly dead. However, we have no estimate of the time of death prior to sampling.

Pathological assessment

On-site assessment of dead mussels indicated they had normal fleshy feet, with no evidence of glycogen depletion. Subsequent examination demonstrated that, at the time of death, gamete production was ongoing, and organs were of normal size and color. A diagnostic report prepared by the National Marine Fisheries Service (Oxford, MD) noted normal trematode infections and no evidence of protozoan, viral, or bacterial-mediated causes of death. The same report noted no evidence of gamete resorption, emaciation, or other indicators of chronic responses to infectious agent. These findings all support a hypothesis that the die-off was acute in origin and was not related to infectious agents.

Analytical chemistry

Five composite mussel samples from the die-off site were analyzed at the Patuxent Analytical Control Facility (Laurel, MD) for 25 common organophosphorus pesticides and six carbamate pesticides. Analyte detection sensitivities were 0.5 to 0.7 μg/g (wet weight) and 1.0 to 2.8 μg/g for each group of compounds, respectively. No pesticides were found. Individual composite samples consisted of either live or dead mussels collected at the Hwy. 1003 bridge, and from the downstream portion of the die-off site near the I-95 bridge (Fig. 1). Mussels were also analyzed for aliphatic hydrocarbons. These analyses were conducted at the Mississippi State Chemical Laboratory, Mississippi State University. Low concentrations of some aliphatic hydrocarbons (0.01–0.08 μg/g) were found, but the concentrations were not considered biologically significant.

Sediment and water samples collected on the day of the die-off were analyzed by the Laboratory Section of the North Carolina Division of Environmental Management in Raleigh according to U.S. Environmental Protection Agency (EPA) protocols for analysis of water and wastewater [10]. None of over 200 target analytes, including base, neutral, and acid extractables, chlorinated pesticides, and organophosphorus pesticides, chlorophenoxy acid herbicides, and volatile organic compounds, were detected.

Cholinesterase activity assessment

We determined cholinesterase activity in live *Elliptio complanata* collected at the Hwy. 1310 bridge, 10 km upstream from the die-off; at the Hwy. 1003 bridge, in the uppermost reach of the die-off area; and at the Hwy. 48 and I-95 bridges, about 7 km downstream from the main area of the kill. Adductor muscle proved to have the richest source of cholinesterase activity in the upstream "reference" samples and therefore was used as the tissue of choice for these assessments. Cholinesterase (ChE) activities were determined by the basic spectrophotometric procedure described by Hill and Fleming [11], employing acetylthiocholine iodide as substrate, a temperature of ~22°C, and a wavelength of 405 nm. Some modifications were made to facilitate work with muscle tissue: (a) Anterior adductor muscle was minced with a scalpel and then homogenized in 10 times its weight of Tris buffer. The homogenate was centrifuged to separate floating fibrous materials. The sample for ChE assay was collected from the clear liquid layer between the pellet at the bottom and the frothy foam at the top of the centrifuge tube. (b) We increased the amount of homogenate used to 200 μl (10 times more than recommended to assay brain and plasma cholinesterase [11]) to compensate for relatively smaller amount of ChE activity in muscle tissue.

Cholinesterase activity for reference samples averaged 1.41 μmol substrate hydrolyzed g^{-1} min^{-1} (SD = 0.40, $n = 7$). Cholinesterase activity in adductor muscle from the four specimens collected at the Hwy. 1003 bridge was depressed 59, 65, 83, and 89% (mean depression, 73%) compared to upstream reference samples. Downstream near the I-95 bridge, cholinesterase activity was depressed 42, 65, 65, 65, and 89% ($n = 5$; mean depression, 65%) relative to reference samples. The observed depression of ChE activity is consistent with a diagnosis of anticholinesterase poisoning of the type commonly produced by organophosphorus and carbamate pesticides [11].

DISCUSSION

Depressed cholinesterase activity in a variety of biological tissues is indicative of exposure to anticholinesterase compounds. In experiments conducted subsequent to this die-off investigation we found that ChE activity in adductor muscle of *E. complanata* responded in a dose-related fashion to both organophosphate and carbamate pesticides. Mussels involved in the Swift Creek die-off exhibited depressed ChE activities similar to those observed in our laboratory experiments. Thus, our finding of depressed ChE activities in mussels from the die-off is consistent with a diagnosis of anticholinesterase poisoning. Organophosphate and carbamate pesticides are the most commonly reported causes of anticholinesterase poisoning in environmental samples. However, confirmation of organophosphate and carbamate poisoning requires identification of these chemicals in tissues or intestinal contents.

Chemical analyses of mussel tissue did not yield detectable concentrations of organophosphate or carbamate pesticides. Failure to detect these pesticide groups in poisoned, moribund animals is common because organophosphates and carbamates are rapidly metabolized and excreted. The analytical detection sensitivities used on our samples also were high relative to water concentrations that induced depressed ChE activity in our laboratory tests, suggesting that the techniques may not have been adequate to detect pesticide concentrations in soft tissues. Thus, we believe that the failure to find pesticide residues in mussels does not preclude a diagnosis of pesticide poisoning. The absence of detectable concentrations of pesticides in the water column at the kill site may also have been related to detection sensitivities, or perhaps to the transient nature of pesticide pulses flowing downstream in the water column.

We conclude from the ChE determinations, acute nature of the deaths, absence of other identified causative factors, and extent of agricultural land use in the watershed that the mussel die-off was caused by an anticholinesterase compound. The most likely compounds are organophosphorus or carbamate pesticides. This is the first case reported in which cholinesterase-inhibiting compounds have been implicated in a die-off of wild populations of freshwater mussels.

Although we present reference ChE activity levels for adductor muscle, our subsequent work has demonstrated that sample preparation techniques can affect cholinesterase activity. We recommend against using our ChE activity levels as baselines for other studies because of the variability that comes with sample handling and preparation. Reference samples collected, handled, and assayed exactly the same as die-off samples should be used to establish baseline activity values for each study or investigation of freshwater mussels.

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