Fleming et al 1993

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 1000 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 in a stream reach dominated by forestry and agriculture during a period of low flow and high water temperature. Pathological examinations did not show any abnormalities and indicated that the die-off was an acute event. Chemical analyses revealed no organophosphorus or carbamate pesticides, or aliphatic hydrocarbons. Cholinesterase activity in adductor muscle from specimens collected at the kill site and downstream was depressed 73 and 65%, respectively, in comparison with upstream reference samples. The depression is consistent with a diagnosis of anticholinesterase poisoning. This is the first documented case ~~reported~~ in which cholinesterase-inhibiting compounds have been implicated in a die-off of freshwater mussels.

Introduction

There are about 300 species and subspecies of freshwater mussels in North America. ~~About~~ Nearly one half of these have become extinct, endangered or have populations that have declined to the point that Federal protection may be necessary (Neves in press). National trends are mirrored in North Carolina, ~~a state~~ with approximately 70 species of freshwater mussels, ~~to~~ including four species that are Federally-listed as endangered and 13 species which are candidates for Federal listing.

Early work has suggested that siltation, pollution, commercial harvest, and dams were important issues in the decline of freshwater mussel fauna in the eastern half of United States (van der Schalie 1938; Fuller 1974; Havlik and Marking 1987). No doubt, some of these factors acted insidiously, altering sediment and water quality characteristics ~~thus leading~~ to gradual reductions in recruitment and survival of vulnerable species. Mussel die-offs, episodic events that may involve hundreds to thousands of mussels dying during a short period of time (days to weeks) have increased in frequency since 1982 (Neves 1987). Neves (1987) 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 group of fauna. The etiology of these die-offs remain largely unknown. For example, of 16 die-offs reported by Neves (1987), in only two could possible causative factors be identified.

Die-off Event

~~At 10:30 hours~~ 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 (Figure 1). The Eastern elliptio (*Elliptio complanata*) was one of the more common species in the stream area and comprised the largest portion of the die-off. Among the estimated 1000 dead and moribund mussels of several species, there were about 111 Tar spinymussels (*Elliptio steinstansana*), a Federally-listed endangered species. No vertebrates, ~~including fish,~~ were found dead at the site.

At 17:30 hours 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 at ~10:00 hours on July 31 at Hilliardston, North Carolina, the closest ambient monitoring station on Swift Creek, approximately 10 km upstream. Water flow at the Hilliardston station on Swift Creek on August 3 was only about 19% (31 cfs) of the average daily flow (161 cfs) due to drought conditions that existed during the summer of 1990 in eastern and central North Carolina. The 31 cfs flow on August ~~4th~~ was the lowest recorded during the period October 1, 1989 to September 30, 1990 (Ragland et al. 1991).

The die-off occurred in a stream reach dominated by forestry and agriculture, with few industrial or urban discharges. The principle crops in the area were sweet potatoes, cotton, tobacco, corn, and soybeans. Lannate (a. i., methomyl) and 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 (Hoeppner, unpubl. report). The county extension agent reported little use of agricultural chemicals in the vicinity during August.

There were 8 permitted point-source discharges into Swift Creek, but only 2 upstream of the die-off site. The permitted discharges upstream of the die-off site were for treated domestic wastewater, with discharge volumes of 0.0028 and 0.004 million gallons per day. These discharges equated to 0.01 percent of average daily flow and 0.2 percent of the 7Q10 flow.

Two 5-gallon containers labeled hydraulic fluid were found on the die-off site; residual contents of these were saved for analyses. An agricultural irrigation pumping station was located about 1 km upstream from the upstream reaches of the die-off. A fishery researcher on-site eight days prior to the die-off observed an individual pumping water from the creek and the presence of six to eight containers of hydraulic fluid and pesticides.

Pathological Assessment

On-site assessment of dead mussels indicated that mussels 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 (Kern, pers. comm. 1990). These findings all support a hypothesis that the die-off was acute in origin and was not related to infectious agents.

Analytical Chemistry

Mussels of two species, representing living and dead individuals at the time of collection, were analyzed for 25 common organophosphorus pesticides and six carbamate pesticides at the U.S. Fish and Wildlife Service's Patuxent Analytical Control Facility in Laurel, Maryland. Samples were analyzed via capillary column gas chromatography with a flame photometric detector for organophosphorus pesticide determinations and nitrogen phosphorus detector for carbamates. The limit of detection was 0.5 µg/g (wet weight) for organophosphorus pesticides and 1.0 µg/g for all carbamates except methomyl. The limit of detection for this analyte was adjusted to 2.0 µg/g based on recoveries in spiked samples. Precision and accuracy, as determined by analyses of spiked samples and duplicates, were acceptable for all analytes.

Mussels were also analyzed for aliphatic hydrocarbons at Mississippi State Chemical Laboratory of Mississippi State University. Analyses were performed by capillary column gas chromatography with a flame ionization detector and analyte confirmation by mass spectrometry. Lower limits of detection were 0.01 µg/g for individual analytes.

None of five mussel samples contained detectable concentrations of organophosphorus or carbamate pesticides. Concentrations of aliphatic hydrocarbons in the five mussel samples ranged from 0.01 to 0.08 µg/g and are not considered biologically significant.

Fluid from the containers found on-site consisted of water and an oily substance which were analyzed for aliphatic hydrocarbons and organophosphorus and carbamate pesticides. Chemical analysis confirmed the presence of oil in drum contents, but no organophosphorus or carbamate pesticides.

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. EPA 600 Series Methods for Analysis of Water and Wastewater. Sediment was analyzed for base, neutral, and acid extractables, chlorinated pesticides, and organophosphorus pesticides. In addition to these analyses, the water sample was assessed for chlorophenoxy acid herbicides and volatile organic compounds. None of over 200 target analytes in these analyses were present above method detection limits.

Cholinesterase Activity Assessment

how many?

We determined cholinesterase activity in Eastern elliptio mussels collected upstream from the die-off (10 km), at the upper most reach of the die-off, and 7 km downstream. The 7 km downstream site harbored some dead mussels; areas further downstream were not searched. 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. We used standard spectrophotometric techniques (Hill and Fleming, 1982) modified only slightly from those used for diagnosing vertebrate poisonings by cholinesterase-inhibiting pesticides. The modifications were: 1) anterior adductor muscle was minced with a scalpel and then homogenized in 10 times its weight of Tris buffer. The homogenate was then centrifuged to separate floating fibrous materials. The sample was obtained from the liquid layer between the pellet that formed at the bottom of the centrifuge tube and a frothy foam at the top of the tube; 2) we used 200 ul of this homogenate for the assay, 10 times more than that recommended by Hill and Fleming (1982) for vertebrate brain and blood.

activity in adductor muscle from specimens collected at
nstream was depressed 73 and 65% respectively in comparison with
igure 2). The depression is consistent with a diagnosis of
oisoning of the type commonly produced by
and carbamate pesticides (Hill and Fleming 1982).

on of organophosphate and carbamate poisoning requires
nese chemicals in tissues or intestinal contents. Analytical
: detect either pesticide group in the mussels. However, failure to
icide groups in poisoned, moribund animals is common
hosphates and carbamates are rapidly metabolized and
tical chemistry detection limits (organophosphorus pesticides
amates = 1.0 ppm) applied to these samples were
ove concentrations of pesticides in water that we subsequently
significantly inhibit cholinesterase activity in *E. complanata*
., manuscript in prep.). Thus, the failure to find pesticide
n the analytical sensitivities, does not preclude a diagnosis of
soning.

0.5 ug/g
1.0 ug/g

nclude from the cholinesterase determinations, acute nature of
bsence of other identified causative factors, and extent of
land-use in the watershed, that the mussel die-off was caused by
nesterase agent, most likely an organophosphorus or carbamate
This is the first case reported in which cholinesterase-inhibiting
s have been implicated in a die-off of wild natural populations of
mussels.

Fourteen species of freshwater mussels are currently found in Swift including the endangered Tar spinymussel and three species that are sed for federal listing (Alderman et al. 1993). The importance of the Creek to the Tar spinymussel cannot be overstated because the Swift population is the largest of three remaining populations, all of which in the Tar River drainage in eastern North Carolina (U.S. Fish and fe Service 1992).

Is this paragraph needed? It appears unsuitable to introduce discussion

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Acknowledgements

ubstantial technical assistance in this investigation was provided by Dr. Fred
Kern of the National Marine Fisheries Service who conducted the
pathological examinations. Tim Donnelly of the North Carolina Division of
Environmental Management coordinated evaluation of site surface water
and sediment. Chemical analyses of mussels were funded under USFWS
Regional Study Identifier RTO67-90-R4. We thank _____ and
_____ for providing helpful reviews of the manuscript.

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

Figure 2. Cholinesterase activity (μmoles activity min^{-1} g^{-1}) of adductor muscles of moribund Eastern elliptios (*Elliptio complanata)* collected at the site of an ongoing freshwater mussel die-off in Swift Creek, Nash County, NC, August 1990. Samples were collected in the upper stream reaches of the die off, and 7 km downstream, but still within the area where dead mussels were observed. Reference samples were collected 10 km upstream.

Figure 1 in prep.

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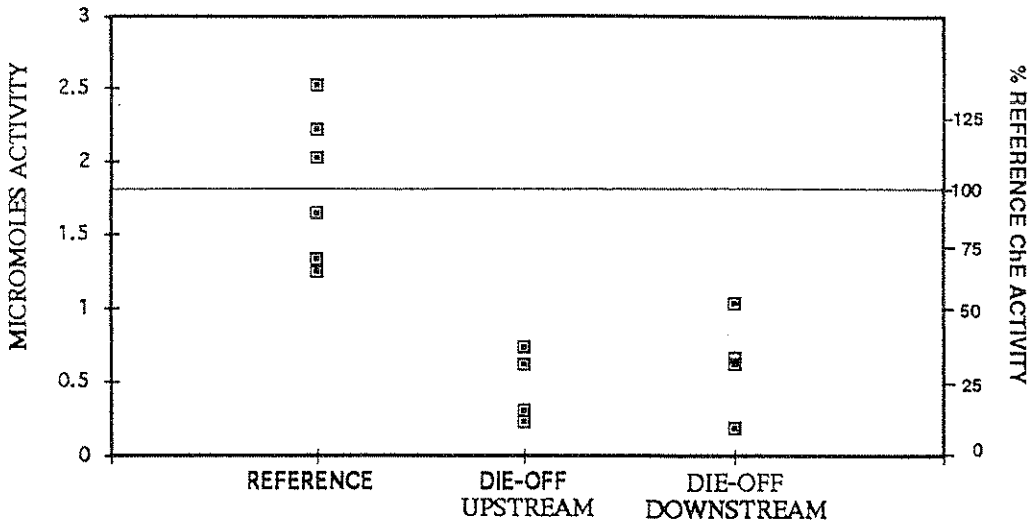
to be included later.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REFERENCE
DIE-OFF UPSTREAM
DIE-OFF DOWNSTREAM

Figure 2

United States Department of the Interior

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TAKE PRIDE IN AMERICA

September 20, 1993

MEMORANDUM

TO: Dick Neves

FROM: Jim Fleming

SUBJECT: Manuscript review

I would appreciate a technical review of the enclosed manuscript, intended for Toxicology and Environmental Chemistry (TEC). If you want to discuss any aspects of the manuscript, please give me a call. Some revisions of the literature cited are required to conform to TEC style.

Please mail your comments to me at the North Carolina Unit, Campus Box 7617, NCSU, Raleigh, NC 27695-7617.

Thanks.

Jim