

to the Washington State Department of Fish and Game for supplying the fish necessary for the testing program.

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The Effects Of A 2,4-D Application On The Biota And Water Quality In Currituck Sound, North Carolina¹

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ABSTRACT

On June 4, 1968 an application of a 20% (acid equivalent) formulation of the butoxyethanol ester of (2,4-dichlorophenoxy)acetic acid (2,4-D) herbicide was conducted to 200 acres of Eurasian watermilfoil (*Myriophyllum spicatum* L.) in Currituck Sound, North Carolina. No acute adverse effects on fish and other organisms were observed. No water samples contained residues exceeding 0.10 ppm. Reduction of Eurasian watermilfoil was estimated at 95% and subsequent re-establishment of native plants was considered to be of significant benefit to waterfowl. It is conceivable that reinfestation by Eurasian watermilfoil could have been reduced and possibly prevented through total treatment of the Sound.

INTRODUCTION

Severe infestation of important fish and wildlife habitats by obnoxious aquatic plants has become a concern of agencies interested in the use and development of these water resources. Such plant infestations seriously curtail fish and shellfish production, crowd out desirable waterfowl food plants, impede recreational uses, promote excessive siltation and greatly reduce the value and public use of the water areas.

The infestation and rapid spread of Eurasian watermilfoil over the past 10 years, during which time it has invaded and become established in many thousands of acres of eastern inland waters, has been studied extensively, but application of control measures has had limited success.

This aggressive plant grows in dense stands that completely dominate the aquatic flora and seriously affect fisheries and wildlife in some of our most productive estu-

aries. Eurasian watermilfoil now has been reported from 18 states. It occurs on at least 7 of the 43 coastal National Wildlife Refuges. The most extensive infestation is in Chesapeake Bay which is an important habitat for valuable commercial fish and shellfish. Many of the areas which have become infested with Eurasian watermilfoil are among the heaviest producers of recreational fishing.

The Natural Resources Institute of the University of Maryland examined a 340 square mile area of Chesapeake Bay, centering on the Bay Bridge, in the summer of 1962. The survey found that the area supported 230,000 angling trips (1.1 per acre) and estimated the taking of 1,751,000 fish weighing about 1,807,000 pounds. The catch consisted of 22 species about one-half of which was perch and one-quarter striped bass. Residue studies of shellfish where 2,4-D was used for Eurasian watermilfoil control indicate that shellfish, including oysters and soft-shelled clams, accumulate and lose 2,4-D related to the treatment. If shellfish are to be harvested from a treated area, a waiting period of 2 to 3 months is recommended (6).

Currituck Sound. Eurasian watermilfoil was first reported in North Carolina in 1959 at the Pea Island National Wildlife Refuge south of Currituck Sound. In 1962 this plant was eliminated by the intrusion of sea water and did not become a problem. First reports of Eurasian watermilfoil in Currituck Sound were made in the summer of 1965, but positive identification was not made until October of that year. There were approximately 100 acres in an infestation stage as a loosely woven blanket several feet thick. Approximately 500 to 1,000 additional acres contained the plant to some degree. By the summer of 1966, rapid spread of Eurasian watermilfoil resulted in an estimated 8,000 acres of heavy infestation, and the plant was established in an additional 67,000 acres.

Treatment Areas. Currituck Sound covers an area of approximately 97,000 surface acres. Average water depth is 5.4 ft with more than 80% less than 7 ft Eurasian watermilfoil was generally distributed over the area at the beginning of the experiment. For purposes of this study four 50-acre areas infested by Eurasian watermilfoil were selected for treatment: a. Swan Island-No. 1; b. Raccoon Bay-No. 2; c. Lighthouse Bay-No. 3; d. Parkers Creek-No. 4.

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During treatment, area boundaries were marked with flags to guide the helicopter pilot and markers were maintained throughout the period of observation.

METHODS AND MATERIALS

Treatment. On June 4, 1968 10 tons of 20% (acid equivalent) 2,4-D butoxyethanol ester granular herbicide were applied by helicopter at the rate of 100 lb per acre to 200 acres. This method gave excellent distribution, and the 2,4-D pellets were observed to be uniformly spread over the water surface.

It was shown that the 2,4-D nonvolatile esters, impregnated on attaclay granules and applied at a rate of 20 lb acid equivalent per acre, were effective for control of Eurasian watermilfoil (1, 4, 5, 7, and 8). In areas subject to water movement, the butoxyethanol ester of 2,4-D gives best results. Crowell and Steenis (2) in preliminary studies found that the butoxyethanol ester of 2,4-D was effective for chemical control. Time and concentration studies have since verified these observations (3).

Fish Samples. A fish population survey was conducted 3 weeks prior to treatment. Other population evaluations were made at 1 day and at 1, 2, 11, 24, 40, and 50 weeks following treatment. Surveys were taken with a 56-ft bag seine and three 100-ft gill nets. The bag seines proved to be more effective in catching fish during the 2 weeks following treatment as extensive plant infestation seriously interfered with the use of gill nets. Plant mats had dissipated after 2 weeks and gill netting became the more efficient method of collecting.

Although plant reinfestation was relatively rapid in most areas, adequate sample sites remained sufficiently weed-free to permit effective use of gill nets throughout the remainder of the study.

One sample of fish for residue analysis was collected at the time the pretreatment population survey was conducted. Since all study areas had similar histories of pesticide usage, a pretreatment fish sample from Area 1 was considered representative of levels in fish from all areas.

Post-treatment fish samples for residue analysis were collected at intervals of 24, 48, and 72 hr; 4, 6, 8, 10, and 13 days; and 2 and 3 weeks. In areas 2, 3, and 4, fish were collected by hook and line until the plants affected by treatment had sunk below the water surface sufficiently to permit the setting of gill nets. Individual specimens were identified, wrapped in aluminum foil, and placed in air-tight plastic bags. They were placed immediately in an ice chest which contained dry ice and later stored in a frozen food locker.

Benthic Samples. Sampling for benthic organisms was conducted 3 weeks prior to treatment to determine species abundant enough for residue analyses. The largest concentration of macroinvertebrates inhabited the dense plant growth along the shore. Collection of post-treatment benthic samples followed the same general schedule as for fish samples. The sweep net proved to be the most effective sampling device. A standard procedure of 6-ft sweeps was used to obtain approximately a 25-g sample necessary for

residue analysis. Benthic organisms for residue analysis were packaged as one sample from each treatment area and frozen in the same manner as the fish samples.

Plankton Samples. Plankton samples were collected from each study area 3 weeks before treatment. Post-treatment samples were taken at intervals of 1 day and 1, 2, 11, 24, 40, and 50 weeks. Each sample was collected by straining 50 gal of water through a number 20 plankton net and preserving the resulting concentrate in a vial containing 5% formalin solution.

Water and Mud Samples. Chemical and physical analyses of the water in each of the study areas were conducted routinely. Dissolved oxygen, pH, total calcium, and water temperature were measured. Water samples for 2,4-D residue analysis were collected prior to treatment and at intervals of 24, 48, and 72 hr; 4, 6, 8, 10, and 13 days; and 2 and 3 weeks following treatment. Each sample consisted of a composite of smaller samples taken at various locations throughout each study area. Water samples were collected in quart-glass jars, labeled and shipped to the laboratory within 48 hr following collection. A preservative (25 ml acetone) was used in each jar in accordance with instructions received from the laboratory analyzing the samples.

Bottom muds for residue analysis were collected at the same time as the water samples. The mud samples were taken from the top layer (1 to 2 inches) with a metal bottom scoop. A composite sample of one quart was collected from each study area, placed into an air-tight plastic bag, labeled and immediately stored on dry ice.

RESULTS AND DISCUSSION

Observations on Eurasian Watermilfoil. Twenty-four hr following treatment, erect seed stems and heads of the target plant, which usually extend from 4 to 6 inches above the surface of the water, had fallen over and lay flat. Forty-eight hr following treatment, 2,4-D injury was apparent, and at 72 hr, the plants had become soft and were beginning to deteriorate. Nine days following treatment, the plant mats were sinking and were 18 to 24 inches below the surface of the water. Mats were beginning to break up. Due to changing "wind tides" and drifts, effects of the herbicidal treatment were observed in Eurasian watermilfoil as much as 50 yards outside the treated areas.

On 23 July, 7 weeks following treatment, the watermilfoil in Areas 2 and 4 had been eliminated. In Areas 1 and 3 it had been reduced by about 95% (Table 1). Dragging the bottom with metal forks yielded little deteriorating plant material, and it was assumed that most of the growth had disintegrated with small amounts possibly drifting into adjacent uncleared areas, or to shore. Other native aquatic plants, including sage pondweed (*Potamogeton pectinatus* L.), southern naiad (*Najas guadalupensis* (Spreng. Morong), redhead grass (*P. perfoliatus*), and water celery (*Vallisneria americana* Michx), appeared in areas where Eurasian watermilfoil had been reduced. Water celery, which was present in trace amounts in Areas 1 and 4 prior to treatment, did not appear to have been affected by the 2,4-D treatment. Observations on 2 September 1968 and 18 June 1969 indi-

TABLE 1. AQUATIC WEED OBSERVATIONS OF AREAS TREATED FOR EURASIAN WATERMILFOIL, CURRITUCK SOUND, NORTH CAROLINA, FOR PRE-TREATMENT GROWTH AND POST-TREATMENT CONTROL AT 7 WEEKS, 3 MONTHS AND 1 YEAR AFTER TREATMENT.

Aquatic Plant Growth in Treatment Areas ^a																
Plant Species	Area 1 Swan Island				Area 2 Raccoon Bay				Area 3 Lighthouse Bay				Area 4 Parkers Creek			
	Date Observed ^b															
Common Name	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d
Eurasian watermilfoil	H	T	M	H	H	-	-	T	H	T	T	M	H	-	T	H
Chara	-	-	T	T	-	-	-	-	-	-	-	-	-	-	T	-
Nitella	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	T
Sago pondweed	-	T	-	T	-	T	T	T	-	T	T	M	-	T	T	-
Redhead grass	-	T	-	T	-	T	T	T	-	-	-	-	-	T	-	M
Pondweed	-	-	-	T	-	-	-	-	-	-	-	-	-	-	T	M
Southern naiad	-	T	-	T	-	T	T	T	-	T	T	T	-	-	T	M
Water celery	T	T	T	T	-	-	-	T	-	-	T	T	T	T	T	-
Elodea	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	T
Widgeon grass	-	-	-	T	-	-	T	-	-	-	T	T	-	-	-	M
Horned pondweed	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-
Coontail	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pickeralweed	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-
Milfoil	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

^aT - Trace, M - Medium growth, H - Heavy growth

^ba, Pretreatment observation, 6/4/68; b, Post-Treatment observation, 7/23/68; c, Post-treatment observation, 9/2/68; d, Post-treatment observation, 6/18/69.

cated that an increasing number of desirable aquatic plants were becoming established in all study areas. Encroachment of Eurasian watermilfoil from adjacent areas caused heavy reinfestation in Areas 1 and 4 and medium reinfestation in Area 3 within 1 year following treatment (Table 1). By 19 June 1969 a small amount of regrowth had occurred in Area 2. This area is protected by two small islands which inhibit introduction of broken fragments of Eurasian watermilfoil by water movement.

Fish. Table 2 lists 13 species of fish found in pre- and post-treatment surveys at each area. Except for *Gambusia*, the remaining 12 species are the most common fish taken by Currituck Sound commercial fishermen in haul seines and gill nets. Several species of minnows were observed swimming in and out of the plant growth during the pretreatment survey. Of the several minnow species observed during the pretreatment survey, *Gambusia* was the

only one taken in sufficient numbers to be included in the study.

Pretreatment samples from the four study areas were comprised of 305 fish representing 11 species. In the seven post-treatment samples 3,363 fish were taken collectively from the four areas of which 1,604 were collected in the 11-week survey, the majority being young-of-the-year fish.

No acute effects to fish from the treatments were observed. It is evident that removal of the bulk of plant infestation permitted an unimpeded influx and movement of fish from surrounding waters and certainly improved accessibility and use by commercial and sport fishermen.

Residues of 2,4-D in whole fish samples from the four study areas are shown in Table 3. Thirty-nine fish samples (38 post-treatment and one pretreatment) were analyzed. Samples for residue analysis contained from one to three specimens. Species of fish used include yellow perch, large-

TABLE 2. NUMBER OF FISH COLLECTED IN PRE- AND POST-TREATMENT SURVEYS AT STUDY AREAS IN CURRITUCK SOUND, NORTH CAROLINA, 1968.

Common Names	Number of Fish Collected							
	Pretreatment			Post-treatment				
	1 Day	1 Week	2 Weeks	11 Weeks	24 Weeks	40 Weeks	50 Weeks	
Longnose gar	8	6	3	16	28	1	3	4
Chain pickerel	12	2	5	12	18	3	0	6
Carp	8	13	29	40	94	16	56	64
Brown bullhead	8	4	7	5	236	6	15	47
Channel catfish	0	13	11	29	49	3	12	17
American eel	4	18	2	8	118	12	24	5
Mosquitofish	202	31	11	20	409	114	306	212
White perch	37	13	43	135	29	16	25	64
Pumpkinseed sunfish	10	16	18	30	116	21	24	90
Bluegill	7	13	32	20	284	12	18	27
Largemouth bass	6	2	15	23	176	12	24	33
Yellow perch	0	0	0	62	44	5	18	28
Southern flounder	3	0	0	0	3	0	1	0
TOTAL	305	131	176	400	1,604	221	526	597

TABLE 3. 2,4-D RESIDUES IN FISH FLESH FROM FOUR STUDY AREAS IN CURRITUCK SOUND, NORTH CAROLINA, 1968.

Date Collected	Time Collected	2,4-D Concentration in PPM and Fish Species Sampled ^a			
		Area 1	Area 2	Area 3	Area 4
05/14/68	Pretreatment	<0.10 PS			
	Post-treatment				
06/05/68	24 hr	<0.10 PS	<0.10 BB	0.18 WP	0.17 WP
06/06/68	48 hr	<0.10 WP	<0.10 CC	<0.10 YP	<0.10 WP
06/07/68	72 hr	<0.10 PS	^b	^b	0.23 WP
06/08/68	4 days	<0.10 WP	<0.10 BG	0.11 CC	0.22 YP
06/10/68	6 days	<0.10 PS	<0.10 BG	<0.10 WP	0.19 CC
06/12/68	8 days	0.24 BB	<0.10 BG	<0.10 YP	<0.10 WP
06/14/68	10 days	0.16 PS	<0.10 WP	0.12 WP	0.18 WP
06/17/68	13 days	0.21 WP	<0.10 WP	<0.10 WP	0.18 WP
06/18/68	2 weeks	<0.10 PS	<0.10 PS	<0.10 PS	0.17 WP
06/25/68	3 weeks	<0.10 BB	<0.10 BG	<0.10 YP	<0.10 WP

^aLetters indicate fish species used in sample as shown below; one to three fish per sample.

^bSamples lost in transit.

PS - Pumpkinseed sunfish
WP - White perch
CC - Channel catfish

BB - Largemouth bass
BG - Bluegill sunfish
YP - Yellow perch

mouth bass, pumpkinseed, channel catfish, white perch and bluegill. 2,4-D residues in the pretreatment samples were at some level below their detectable limit of 0.10 ppm. The highest residue concentration (0.24 ppm) was in a largemouth bass from Area 1, 8 days following treatment. No species of fish seemed to be most active in 2,4-D accumulation.

Benthic Organisms. Benthic organisms selected for residue analysis were glass shrimp (*Palaemonetes* sp.), damselfly nymphs (*Ischnura* and *Enallagma* sp.), and scuds (*Gammarus* sp.). These organisms were selected because of their abundance and ease of collection with the sweep net. Blue crabs (*Callinectes sapidus* Rathbun) were collected from Areas 2, 3, and 4 with gill net. The 6-ft sweep net samples taken during each survey yielded between 150 to 200 damselfly nymphs except during the 2 September 1968 survey when each net sample yielded from 20 to 25 specimens. This low number of damselfly nymphs per net sample was

attributed to a recent hatch. The dense shoreline plant growth and calm water areas were covered with empty nymph cases.

One pretreatment and 30 post-treatment samples of benthic organisms were analyzed for 2,4-D residues as indicated in Table 4. Samples of benthic organisms were a mixture of the various species selected for analysis. Blue crabs were analyzed separately. The highest residue (0.23 ppm) was recorded from organisms collected from Area 1, 24 hr following treatment. All residues in blue crabs were below the detectable limit of 0.10 ppm.

Plankton. Nineteen plankton samples were analyzed during the study, of which four were pretreatment samples. Variations in number and species of organisms between the samples were minor and could have been normal seasonal fluctuations. Since there is no data available relative to plankton populations in Currituck Sound, only data from pretreatment samples could be used for comparison.

TABLE 4. 2,4-D RESIDUES IN BENTHIC ORGANISMS (GLASS SHRIMP, DAMSELFLY NYMPHS, SCUDS AND BLUE CRABS) FROM FOUR STUDY AREAS IN CURRITUCK SOUND, NORTH CAROLINA, 1968.

Date Collected	Time Collected	2,4-D Concentration in PPM			
		Area 1	Area 2	Area 3	Area 4
05/14/68	Pretreatment	<0.10			
	Post-treatment				
06/05/68	24 hr	0.23	0.17	0.13	^a
06/06/68	48 hr	<0.10	0.13	0.12	^a
06/07/68	72 hr	<0.10	0.15	0.21	^a
06/08/68	4 days	<0.10	0.12	0.19	^a
06/10/68	6 days	<0.10	0.11	<0.10	^a
06/12/68	8 days	<0.10	<0.10	0.16	^a
06/17/68	13 days	<0.10	0.14	0.14	<0.10
06/18/68	2 weeks	<0.10	<0.10	<0.10	0.19
06/25/68	3 weeks	<0.10	<0.10	<0.10	<0.10
		Blue Crabs			
06/08/68	4 days		<0.10		
06/10/68	6 days			<0.10	
06/12/68	8 days				<0.10

^aData missing for 24 hr to 8 days from Area 4 is due to insufficient time for collection of benthic samples. This area was considered least important and sampling efforts during this period were concentrated in Areas 1, 2, and 3.

TABLE 5. 2,4-D RESIDUES IN MUD SAMPLES FROM FOUR STUDY AREAS IN CURRITUCK SOUND, NORTH CAROLINA, 1968.

Date Collected	Time Collected	2,4-D Concentration in PPM			
		Area 1	Area 2	Area 3	Area 4
	Pretreatment	<0.10			
	Post-treatment				
06/05/68	24 hr	0.22	0.26	0.52	0.27
06/06/68	48 hr	0.22	0.31	0.50	0.55
06/07/68	72 hr	<0.10	0.38	0.50	0.50
06/08/68	4 days	0.42	0.17	0.51	0.50
06/10/68	6 days	<0.10	0.22	0.60	0.55
06/12/68	8 days	<0.10	0.27	0.20	0.47
06/14/68	10 days	0.27	0.37	0.20	0.65
06/17/68	13 days	0.42	0.26	<0.10	0.55
06/18/68	2 weeks	0.33	0.32	0.20	0.30
06/25/68	3 weeks	0.32	0.20	<0.10	0.27

Water. The physio-chemical analyses were taken daily from 4 through 26 June 1969. The water temperature ranged from 26 to 30 C; dissolved oxygen from 7.8 to 8.5 ppm; pH from 6.7 to 7.2; and total calcium remained constant at 50 ppm throughout the study.

One pretreatment and thirty-three post-treatment water samples were analyzed for 2,4-D. Samples were collected over a 3-week period following treatment. All were below the detectable limit (0.10 ppm). The low residues in the water could have been materially influenced by two factors: (1) increase in water level 1 to 2 ft immediately following treatment, and (2) degradation of sample while in transit to laboratory.

Mud. The 2,4-D residue data from 41 mud samples (40 post-treatment and one pretreatment) are presented in Table 5. The highest concentration (0.65 ppm) was recorded from Area 4 in a sample collected 10 days following treatment. It is difficult to explain the erratic changes in residue levels in mud samples taken during this study. Smith and Isom, (8), reported erratic changes in mud samples following treatment of 2,500 ha of Eurasian watermilfoil with 2,4-D in Watts Bar and Gunter'sville Reservoirs. They found residue levels ranged from 0.24 to 58.8 ppm in mud samples collected 10 months following treatment.

SUMMARY AND CONCLUSIONS

Application of 100 lb per acre of 20% granular 2,4-D butoxyethanol ester herbicide (20 lb acid equivalent) initially reduced Eurasian watermilfoil growth in four study areas in Currituck Sound, North Carolina, by an estimated 95% in June 1968. Plant infestation in approximately 200 additional acres adjacent to the study areas was affected by the treatment. Due to rapid encroachment from adjacent untreated areas, reinfestation by this species was heavy in 50% of the treated areas within 1 year following chemical application and present in "trace" to "medium" densities in the remainder. No acute adverse effects to fish and macroinvertebrate populations which

could be attributed to the treatment were observed. Changes which occurred in the fish population structure following treatment indicate that elimination of heavy plant growths improved the conditions for sampling and/or rendered the areas more habitable for fish. The small number of damselfly nymphs taken during the September 2 survey and the large numbers of spent cases observed indicated a hatch had recently occurred in this species. No changes were detected in the water quality. Accumulation of 2,4-D occurred rapidly in fish flesh, certain macroinvertebrates, and mud from the treated areas. Three weeks following treatment, residue levels in fish and benthos had decreased to a level below the detectable limit of 0.10 ppm. Residue levels in mud samples during the same period persisted above the detectable limit. 2,4-D residues were below the detectable limit (0.10 ppm) in all water samples.

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