

# Changes In A *Myriophyllum spicatum* L. Community Following 2,4-D Treatment

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## ABSTRACT

A community of Eurasian watermilfoil (*Myriophyllum spicatum* L.) in Kitty Hawk Bay, North Carolina, was treated on July 13, 1974 with a granular 20% acid equivalent formulation of the butoxyethanol ester of 2,4-dichlorophenoxyacetic acid (2,4-D) at the rate of 112 kg/ha (100 lb/acre). Native submersed macrophytes associated with the Eurasian watermilfoil stands included widgeon grass (*Ruppia maritima* L.), southern naiad (*Najas guadalupensis* (Sprengel) Mangus.) and sago pondweed (*Potamogeton pectinatus* L.). The Eurasian watermilfoil community thrived under low ambient orthophosphate concentrations. Levels of phosphorus and nitrogen in plant tissues were generally low. Eurasian watermilfoil was eliminated and native species biomass decreased 6 weeks after treatment. Water turbidity increased during the post-treatment period, accompanied by a cyanophytoplankton bloom. Deterioration of Eurasian watermilfoil in isolated coves near the treatment area continued 8 weeks after the initial treatment. Accelerated growth and re-establishment of native submersed macrophytes did not occur during the 1974 growing season. Eurasian watermilfoil and native submersed macrophytes recovered slowly during the 2-year, post-treatment growing season. A successional pattern in the Kitty Hawk Bay treatment area developed with stonewort (*Nitella hyalina* (DC) Ag.) as the pioneer species. By the summer of 1978 a community dominated by Eurasian watermilfoil had developed.

## INTRODUCTION

Eurasian watermilfoil (*Myriophyllum spicatum* L.) was first found in the northern Currituck Sound in 1964, and by 1966 had spread throughout most of the sound (23). Kitty Hawk Bay, just south of Currituck Sound, was heavily infested with Eurasian watermilfoil (hereafter referred to as milfoil) by 1968 (5).

In the summer of 1974 an herbicide program was developed to treat milfoil in maximum use areas (commercial fishing, recreation, channels, docks, etc.) in Kitty Hawk Bay.

This study was undertaken to (a) determine the effectiveness of 2,4-D BEE applied at 112 kg ae/ha in controlling milfoil, (b) determine the effect of the herbicide on certain

native submersed macrophytes, (c) provide a detailed analysis of inorganic nutrients of submersed macrophytes in the Kitty Hawk Bay area, and (d) determine species composition and rate of recolonization of plant communities following 2,4-D treatment.

## STUDY AREA

Kitty Hawk Bay is a shallow protected body of water located in northeastern, coastal North Carolina. The bay is separated from the Atlantic Ocean by a barrier spit extending southward from southeastern Virginia. Oregon Inlet, which marks the end of the spit some 40 km to the south, is the nearest direct connection to the sea. Since this is a shallow inlet with a low exchange volume, the Kitty Hawk Bay area has low salinity (2 to 5 o/oo). At times the salinity may increase due to wind tides and infrequent washovers across the spit. In the areas treated with 2,4-D, the water depth varied from 1 to 2 m. Water turbidity was low and the bottom was easily visible most of the time. The bottom consisted of a firm sand-mud mixture, which became softer and more organic in the coves. All of the bay had a high density of submersed macrophytes, primarily dominated by milfoil. Wind tides caused water levels to fluctuate as much as 50 cm.

## MATERIALS AND METHODS

On July 13, 1974, granular 2,4-D BEE was applied by helicopter on approximately 295 ha of Kitty Hawk Bay at the rate of 112 kg ae/ha. Winds were northeasterly at 16 km/h during application.

In Kitty Hawk Bay, a boundary line was established dividing the study plot into a treatment plot and an adjacent control plot (Control I). Midpoints of 10 randomly selected transects were marked with wooden stakes along this boundary line. Each transect ran perpendicular to the boundary line and was composed of 20 sample points at 25 m intervals. The total original study plot in Kitty Hawk Bay was approximately 500 by 1000 m. Control II (100 by 300 m) was established during the post-treatment study approximately 800 m west of Control I and contained five transects, each with 10 points at 10 m intervals. A square iron quadrat frame (0.1 m<sup>2</sup>) was dropped on the bottom near the sampling point and all plants rooted within the frame were harvested.

Pretreatment biomass determinations were based upon the random selection of four 0.1 m<sup>2</sup> quadrats along each transect in Kitty Hawk Bay giving a total of 40 quadrats, 20 in the treatment plot and 20 in Control I. Post-treatment biomass determinations were as above and collected on post-treatment weeks 6 and 52. The study site was surveyed on July 24, 1976 by skin diving and ten 0.35 m<sup>2</sup> random quadrat samples of submersed macrophytes were taken from the

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Treatment Area and Control I (combined) on August 2, 1978. Whole plants were dug from the quadrats, brought to the laboratory, washed thoroughly, separated and counted by species and then separated into roots and shoots. Samples were centrifuged in a top loading washing machine for 10 min on spin cycle and wet (fresh) weight determined. A 10 to 25 g subsample was randomly selected from each sample and dried at 70 C for 24 h. Ash free dry weight was determined by the combustion of dried samples in a muffle furnace at 550 C for 4 h. Dried plant samples were ground to 20 mesh in a Wiley mill and stored at 5 C prior to chemical analysis.

Samples were prepared for phosphorus and cation analysis by ashing approximately 1 g of dried plant material at 500 C for 4 h followed by dissolving the ash residue in 6 N HCl. The resulting solution was boiled, evaporated, washed, and filtered. The filtrate was diluted to 100 ml with deionized water and stored at 5 C. Phosphorus was determined by the molybdate method (11), calcium and magnesium by atomic absorption and potassium and sodium by flame emission. Nitrogen was determined from dried plant material with a Coleman Nitrogen Analyzer. Surface water samples were collected weekly in all study areas during the summers of 1974 and 1975. Water nutrient analysis was performed in accordance with EPA methods (11).

## RESULTS AND DISCUSSION

Within 15 days of 2,4-D application approximately 600 ha of milfoil were eradicated in Kitty Hawk Bay, although only 295 ha were directly treated. The drift of 2,4-D in Kitty Hawk Bay was over 300 m from the treatment edge as compared to previous reports in Currituck Sound of only 46 m for granular 2,4-D (23). This extensive herbicide drift was most likely due to water currents created by north-easterly winds during application. When milfoil in Control I was affected by the herbicide drift a healthy stand of milfoil, Control II, was established at 15 days post-treatment. Native submersed macrophytes seemed unaffected by the herbicide.

Once milfoil disappeared from the study area, greater wave action stirred the bottom sediments and increased water turbidity. Secchi disc transparency was <0.3 m after the milfoil decline, as compared with >1.5 m before treatment. Native submersed macrophytes began to deteriorate during the third post-treatment week. By the fourth post-treatment week (13 August) a phytoplankton bloom (which also contributed to turbidity) was observed. The bloom was composed primarily of the cyanophytes *Aphanizomenon* sp. and *Anacystis* sp. (14). About the time of this bloom a 30 to 50 ha area of milfoil began dying in Control II and the surrounding coves. This delayed mortality did not display the typical 2,4-D symptoms (13). Deterioration was much slower. Leaves remained attached to the stems as decomposition progressed and some of the plants became coated with what appeared to be an algal slime. Steenis and Stotts (21) and Rawls (18) reported that algal growths on milfoil may be significant in delayed milfoil mortality, since applied herbicides may accumulate in the algae with release later. Wojtalik et al. (24) found that phytoplankton in Tennessee

Valley Authority (TVA) reservoirs sorbed large amounts of 2,4-D and retained them for extended periods. Such 2,4-D dynamics and/or high turbidity may have been factors in the delayed milfoil decline at Kitty Hawk Bay. These late symptoms seemed similar to those described by Bayley et al. (6) which they attributed to "Lake Venice disease."

Studies of milfoil communities treated with 2,4-D in Currituck Sound (23), Chesapeake Bay (18) and TVA reservoirs (24) suggest that native submersed macrophytes survive and increase in abundance following the disappearance of milfoil. Accelerated growth and substantial re-establishment of native vascular submersed macrophytes did not occur in the treated area during the study period. Native macrophyte abundance declined during the first 8 weeks of the post-treatment period. The native macrophyte decline might be attributed to the relatively high concentration of 2,4-D (112 kg ae/ha) used in treatment. By comparison 2,4-D BEE at 135 kg ae/ha killed all submersed vegetation in the lower Potomac River (7) while 2,4-D BEE at 112 kg ae/ha killed only milfoil in Currituck Sound (23).

One year after herbicide application, the Kitty Hawk Bay treatment area was covered with a dense bottom mat of stonewort (*Nitella hyalina* (DC) Ag.). This "rooted" alga had been observed near the study plots during the pre-treatment collection period. Secchi disc visibility had increased to 0.4 m by the summer of 1975. Since stonewort extended only 5 to 10 cm above the substrate, wave dampening effectiveness was limited. Yet, these charophyte beds may function as a pioneer stage of submersed macrophyte community succession by competitive inhibition of phytoplankton blooms through nutrient utilization and by reduction of water turbidity through plankton reduction and substrate stabilization. Young stems of milfoil and native vascular species were observed growing in the stonewort beds.

The initial ash free dry weight (AFDW) of submersed macrophytes in Kitty Hawk Bay was 258.4 g/m<sup>2</sup> of which 95% was milfoil (Table I). Milfoil had disappeared 6 weeks after treatment in all plots and native macrophyte biomass was reduced to 23% of pretreatment levels. In Control II, the total biomass was 76.8 g AFDW/m<sup>2</sup>, of which 94% was milfoil.

The total biomass 1 year after treatment had reached 22.6 g AFDW/m<sup>2</sup> in the Treatment Area and Control I and 33.5 g AFDW/m<sup>2</sup> in Control II. Milfoil only accounted for 3.5% of the biomass in the Treatment Area and Control I and 3.8% in Control II. Stonewort dominated the Treatment Area and Control I (87.6%), while widgeon grass dominated Control II (73.4%). Low biomass of native submersed macrophytes in the post-treatment area confirms the previously mentioned lack of regrowth of native vascular macrophytes in the treated areas.

Two years after treatment (July 24, 1976), Stonewort, widgeon grass and muskgrass (*Chara* sp.) were scattered in the Treatment Area and Control I with isolated shoots of milfoil ranging from 30 to 50 cm long. The community in Control II was similar but stonewort and widgeon grass were more dense and no milfoil was found.

A milfoil community had become re-established in the

TABLE 1. ASH FREE DRY WEIGHT (G/M<sup>2</sup>) OF SUBMERSED MACROPHYTES IN TREATMENT AREA, CONTROL I AND CONTROL II OF KITTY HAWK BAY, BEFORE AND AFTER 2,4, D TREATMENT. EACH VALUE IS THE MEAN  $\pm$  1 STANDARD ERROR OF 20 HARVESTED QUADRATS.<sup>a</sup>

Species	Pretreatment	Post-treatment		
		6 weeks	1 year	4 years
Treatment and Control I				
Eurasian watermilfoil ( <i>Myriophyllum spicatum</i> )	242.9	0.0	0.8	28.8
Widgeon grass ( <i>Ruppia maritima</i> )	7.2	2.9	1.1	TR <sup>b</sup>
Southern naiad ( <i>Najas guadalupensis</i> )	3.5	0.1	0.5	0.0
Sago pondweed ( <i>Potamogeton pectinatus</i> )	4.8	0.6	0.0	0.0
Wild celery ( <i>Vallisneria americana</i> )	0.0	0.0	0.4	TR
Stonewort ( <i>Nitella hyalina</i> )	0.0	0.0	19.8	0.0
Muskgrass ( <i>Chara braunii</i> )	0.0	0.0	0.0	TR
Totals	258.4 $\pm$ 18.2	3.6 $\pm$ 0.6	22.6 $\pm$ 2.4	28.8 $\pm$ 11.1
Control II <sup>c</sup>				
Eurasian watermilfoil ( <i>Myriophyllum spicatum</i> )	—	71.9	1.3	—
Widgeon grass ( <i>Ruppia maritima</i> )	—	3.0	24.6	—
Southern naiad ( <i>Najas guadalupensis</i> )	—	1.9	3.8	—
Wild celery ( <i>Vallisneria americana</i> )	—	0.0	0.4	—
Stonewort ( <i>Nitella hyalina</i> )	—	0.0	3.4	—
Totals	—	76.8 $\pm$ 6.7	33.5 $\pm$ 6.9	—

<sup>a</sup>Post-treatment 4 years is the mean  $\pm$  1 standard error of 10 harvested quadrats.

<sup>b</sup>TR = trace (<0.1 g AFDW/m<sup>2</sup>).

<sup>c</sup>Established after 2,4-D application.

Treatment Area and Control I by August 2, 1978, 4 years after treatment. Mean milfoil biomass was 28.8 g AFDW/m<sup>2</sup> with trace amounts (<0.1 g AFDW/m<sup>2</sup>) of widgeon grass, wild celery, southern naiad and muskgrass (*Chara braunii* Gmelin) present. Hence this community was similar in structure to the one present before 2,4-D treatment in 1974. Its lower biomass was probably associated with increased turbidity and turbulence which resulted in a drastic decrease in milfoil biomass in Coinjock Bay in the northern Currituck Sound in the spring of 1978 (10).

Nutrient concentrations of milfoil, sago pondweed and southern naiad collected in the summer of 1974 are compared with values reported from other studies in Table 2. In most cases, tissue nutrient levels from this study are lower than nutrient levels reported from other locations, particularly with respect to nitrogen and phosphorus.

Surface water samples were collected weekly and analyzed for inorganic nutrients during the summers of 1974 and 1975 in the Treatment Area and Control I. The cations sodium, potassium, calcium and magnesium were found in ratios similar to those in sea water. No clear trends were noted for variations in nitrate and nitrite in the water. Am-

monium increased from a mean of 0.98 ( $\pm$ 0.19 SE)  $\mu$ g-at/l in 1974 to a mean of 20.17 ( $\pm$ 3.12 SE)  $\mu$ g-at/l in 1975. Once biomass was removed by 2,4-D treatment, the increased wave action and turbulence may have caused a greater exchange of ammonium between the sediments and the water column than had existed before treatment. Ortho-phosphate levels remained low with a mean value of 0.18 ( $\pm$ 0.05 SE)  $\mu$ g-at/l in 1974 and a mean of 0.25 ( $\pm$ 0.06 SE)  $\mu$ g-at/l in 1975.

Milfoil was the dominant where phosphate levels were low and predominated with lower tissue phosphate levels than were found in other submersed macrophytes in this system (Table 3). This suggests that milfoil predominance may be due, in part, to lower critical tissue levels of phosphorus in a comparison to requirements for survival and growth. The comparatively low critical tissue concentration of phosphorus for milfoil (0.07%) reported by Gerloff (12) is consistent with the Kitty Hawk Bay studies. These observations suggest that milfoil may outcompete other submersed macrophytes in systems with low phosphorus. In fact, milfoil was re-established as the dominant plant in Kitty Hawk Bay by 1978. The milfoil infestation of Kitty Hawk

TABLE 2. COMPARISON OF MEAN INORGANIC NUTRIENTS OF EURASIAN WATERMILFOIL, SAGO PONDWEED AND SOUTHERN NAIAD FROM VARIOUS SOURCES.

Species and Source	Percent dry weight					
	N	P	Na	K	Ca	Mg
<b>Eurasian watermilfoil</b> ( <i>Myriophyllum spicatum</i> )						
Nelson and Palmer (17)	4.13	0.42	0.75	1.87	2.77	0.74
Umeda and Tamaki (22)	1.51	0.05	—	1.18	1.31	—
Anderson et al. (4)	3.00	0.40	1.20	2.70	0.35	0.05
Boyd (8)	1.57	—	—	—	—	—
Reimer and Toth (19)	2.89	0.50	1.01	1.68	1.31	0.35
Adams and McCracken (2)	—	0.18	—	—	—	—
Gerloff (12)	2.72	0.26	—	0.20	—	—
This study	1.16	0.07	0.90	1.03	0.78	0.29
<b>Sago pondweed</b> ( <i>Potamogeton pectinatus</i> )						
Harper and Daniel (15)	1.98	0.16	—	—	3.00	—
Allenby (3)	—	—	—	—	3.20	—
Reimer and Toth (19)	1.72	0.26	0.60	1.73	2.63	0.30
Adams et al. (1)	—	0.30	0.20	1.33	0.86	0.38
Neel et al. (16)	3.30	0.31	0.40	0.70	1.76	0.26
This study	1.35	0.08	0.77	1.16	0.92	0.28
<b>Southern naiad</b> ( <i>Najas guadalupensis</i> )						
Boyd (8)	3.65	—	—	—	—	—
Davis and Brinson (9)	3.13	0.71	—	—	—	—
This study	2.37	0.14	1.00	3.94	1.04	0.33

Bay may be related, in part, to low available phosphate. However, optimum milfoil growth may be triggered by a combination of nutrient conditions. Stanley and Goode (20) suggested that the micronutrient borate might stimulate milfoil growth in low phosphate areas of TVA lakes.

Long-term control of milfoil in Kitty Hawk Bay with herbicides is unlikely because of the vast stands of milfoil in the area. These beds are the source of vegetative fragments which can lead to early reinfestation of a treated area. Apparently 2,4-D at 112 kg ae/ha is potent enough to severely damage native submersed macrophytes found in Kitty Hawk Bay and, under certain wind conditions, 2,4-D BEE can directly kill up to twice the target area. It is also apparent that milfoil in this system will outcompete native vascular submersed macrophytes in an area denuded following 2,4-D treatment. However, as reported for the Kitty Hawk Bay treatment area, native charophytes may be pioneer species in community succession and eventually be replaced by vascular macrophytes with milfoil dominating.

TABLE 3. SUMMARY OF INORGANIC NUTRIENTS OF EURASIAN WATERMILFOIL, WIDGEON GRASS, SAGO PONDWEED AND SOUTHERN NAIAD IN KITTY HAWK BAY, 1974.<sup>a</sup>

Species	Structure	(n)	Percent ash free dry weight					
			N	P	Na	K	Ca	Mg
Eurasian watermilfoil ( <i>Myriophyllum spicatum</i> )	shoots	17	1.45 d	0.06 e	1.06 bc	1.06 e	0.85 c	0.22 c
	roots	19	1.60 d	0.08 de	0.66 d	1.31 de	1.08 b	0.37 b
Widgeon grass ( <i>Ruppia maritima</i> )	shoots	9	2.24 c	0.13 cd	0.89 dc	3.13 bc	0.99 bc	0.44 ab
	roots	5	1.81 d	0.12 cde	0.87 dc	2.27 cd	0.95 bc	0.45 ab
Sago pondweed ( <i>Potamogeton pectinatus</i> )	shoots	5	1.69 d	0.12 cde	1.12 bc	2.43 cd	1.49 a	0.42 ab
	roots	2	1.88 cd	0.44 a	1.61 ab	2.67 bcd	0.87 bc	0.36 bc
Southern naiad ( <i>Najas guadalupensis</i> )	shoots	10	3.21 a	0.20 b	1.36 ab	6.48 a	1.41 a	0.44 ab
	roots	7	2.86 b	0.16 bc	1.64 a	3.96 b	1.35 a	0.51 a

<sup>a</sup>Values in a column followed by the same letter are not significantly different at the 0.05 level as determined by Duncan's Multiple Range Test. Each value is the mean of (n) replicates.

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