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Effect of an Aerial Application of Carbaryl on Brook Trout (*Salvelinus fontinalis*)

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The eastern spruce budworm (*Choristoneura fumiferana*) is a serious economic pest in the spruce-fir forests of northeastern North America. In the U.S.A. the major problem area is northern and western Maine. In 1954-1979 6 different major chemicals and at least 21 experimental chemicals were applied on up to 3.5 million acres annually in attempts to control the budworm. The early use of DDT for this purpose caused extensive losses of Atlantic salmon, *Salmo salar* L., and brook trout, *Salvelinus fontinalis* Mitchill (KERSWILL 1967). Consequently, DDT was replaced by carbamate or organosphosphorus compounds. Use of these compounds has not resulted in visible fish mortalities (KERSWILL & EDWARDS 1969), but sublethal effects may occur.

In 1975-1980 the most widely used chemical for spruce budworm control in Maine was a carbamate, carbaryl (Sevin-4-oil), usually applied at the rate of 1 lb of active ingredient (AI) per acre. Such applications have been shown to increase downstream drift of aquatic invertebrates, and depress population densities of some species of aquatic invertebrates (COURTEMANCH & GIBBS 1978) and to depress brain acetylcholinesterase (AChE) activity in fish (HULBERT 1978; MARANCIK 1976). In the present study an experimental area was established in which carbaryl was applied at the rate of 0.5 lb AI/acre in two applications spaced 7 days apart. The study was conducted to evaluate the effect of this split application on brook trout mortality, condition factor, food, and brain AChE activity.

MATERIALS AND METHODS

This study was conducted in two small streams located in northwestern Maine (Figure 1)--Little Russell Stream which was within the sprayed area, and Logan Brook, which was outside and upwind of the area, where contamination by spray drift was small or nil. The two streams were similar in size and watershed area, and endemic brook trout (*S. fontinalis*) predominated in the fish fauna of both.

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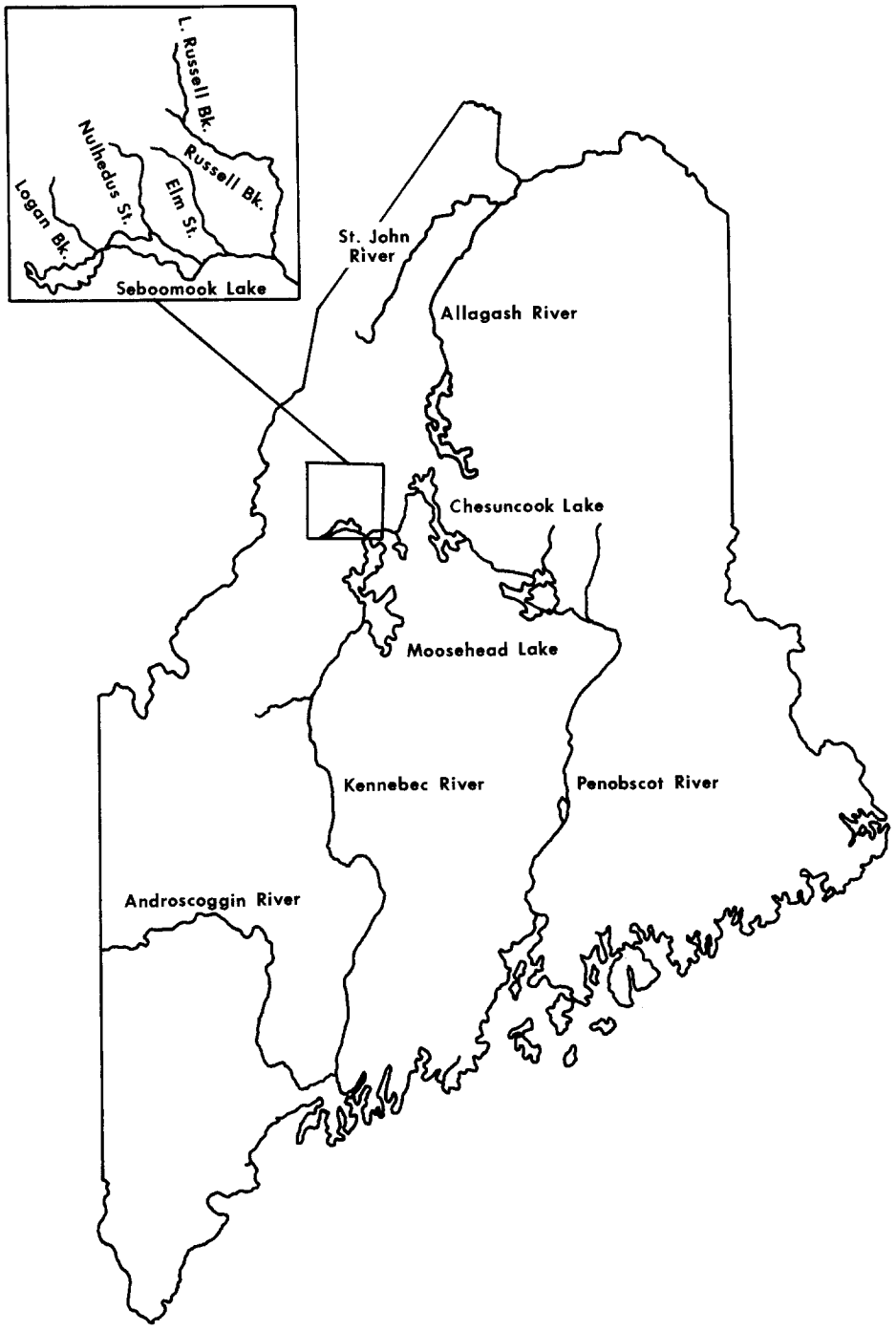

Figure 1. Location of the study area (Seboomook, Somerset and Piscataquis Counties; T5 R15, T5 R16, T4 R15, T4 R16).

Adult brook trout from the treated and control streams were used to measure the impact of the insecticide on brain AChE activity. Five fish of about the same size (mean length 141 mm, range 93-184 mm in Little Russell Stream, 145 mm and 95-200 mm in Logan Brook) were collected by electrofishing from each stream on each sampling date for AChE analysis. Fish were collected 2 weeks, 3 and 1 day before, and several hours, 1, 3 and 5 days after the first spraying (June 7), and several hours, 1 day, 3 days and 4 weeks after the second spraying (June 14). Stream water temperature was measured on each collection date with a pocket thermometer. The fish collected were placed in an insulated ice chest with 80 L of stream water and kept alive until analyzed. AChE analysis was performed within 2 h after collection.

For AChE analysis, fish were killed by flexure of the spinal chord behind the head. They were immediately weighed and measured and the brain was removed and weighed to the nearest 0.01 g. The entire brain was homogenized in a glass tissue grinder in pH 8.0 Tris buffer at the ratio of 100/mg brain mL of buffer. The homogenate was placed on an ice bath during analysis. The AChE analysis method was a modification of the Ellman reaction (ELLMAN et al. 1961) described by HILL (1979). In a further modification to this technique all reagents were held at 21°C in a water bath, thus precluding any temperature-induced changes in enzyme activity during analysis. Two analyses were performed on the brain homogenate from each fish. A condition factor (CF) was calculated for each fish by the equation $CF = \frac{W}{L_3} \times 10^5$ where W = weight in grams, and L = total length in millimeters.

After AChE analysis, whole stomachs were removed from each fish and preserved in 10% buffered formalin. In the later food analysis, stomach contents were emptied into a tared watch glass, excess liquid was removed by blotting with a paper towel, and the wet weight of total contents was obtained. The contents were then separated into individual items, and organisms were enumerated by taxonomic order. Quantity of food was expressed as percent of total body weight.

An *in situ* toxicity test was performed in the control and treated stream with young-of-the-year brook trout collected from Russell Stream by electrofishing. About 50 fish were placed in a screened box (50 x 50 x 30 cm), which was submerged in water with a moderate current in each stream. Any dead fish were counted and removed on the days when adult fish were collected from the stream for AChE analysis.

Data on spray application times and precipitation were obtained from the Maine Forest Service. The rain gauge was located at Pittston Farm, 7 mi west of Logan Brook.

The data for AChE activity, condition factor, and percent body weight as food were tested for statistical significance. The data met all assumptions for analysis of variance (the arcsin square root transformation was applied to the percent data). The

means for treated and control streams were compared for each sample date by partitioning the overall treatment sum of squares and degrees of freedom into orthogonal, a priori comparisons, which were then compared by using F-tests (SOKAL & ROHLF 1969).

RESULTS

Mean fish brain AChE activity was significantly (P=0.05) lower (15 to 34%) in fish from Little Russell Stream than in fish from Logan Brook (the control stream) on June 10 and 14, but the depression disappeared by the next sampling period, 24 or 48 h later (Figure 2). Condition factor was consistently higher in fish from Little Russell Stream fish than in those from Logan Brook (Figure 3). The difference was significant on June 4, 6, 7, 12, 14, 15, and 17. Food weight as percent of body weight was significantly higher in Little Russell Stream fish on June 6, 14, and 15 (Figure 4). Only Coleoptera, Diptera, Ephemeroptera, Trichoptera, and terrestrial insects occurred regularly in the diet in appreciable numbers (Table 1). Statistical tests were not performed, but the number of Diptera, Ephemeroptera, and Trichoptera increased in fish from the treated stream on and sometimes following the date of spray application. Terrestrial insects increased only after the second spraying.

The results of the in situ toxicity test indicated no mortalities associated with spray application. In Little Russell Stream there were three dead fish on June 6 (before the first spraying) and one on June 8 (one day after first spraying). There were no further mortalities by the date of release, June 17. No mortalities were observed in the Logan Brook cage by June 10 (the cage was stolen between June 10 and 12).

DISCUSSION

The AChE values obtained for unexposed brook trout in this study--8.4 to 10.5 μmole $g^{-1}min^{-1}$--agree well with those in previous studies: MARANCIK (1976) reported values of 6.5 to 9.5 and ZITKO et al. (1970) values of 6.8 to 11.7 μmoles $g^{-1}min^{-1}$. A moderate nonsignificant depression in AChE activity was observed on the day of the first spraying, when weather was clear, calm, and warm. Under these conditions spray drift off target would be minimized, and there was no spray residue on stream-side rocks or vegetation. The AChE depressions of 15 to 34% observed on June 10 probably were a result of pesticide being washed into the stream after a heavy rainfall on June 9 and 10. On the day of the second spraying the weather was overcast, windy, and cold, and there was spray residue on stream-side rocks and vegetation.

The amount of AChE depression found in this study after application of carbaryl at 0.5 lb AI/acre was similar to the 11 to 38% found in brook trout in studies where the application rate was 1 lb AI/acre (MARANCIK 1976; HULBERT 1978). However, the AChE depression in fish of Little Russell Stream disappeared within 24 h, whereas HULBERT (1978) reported that it persisted

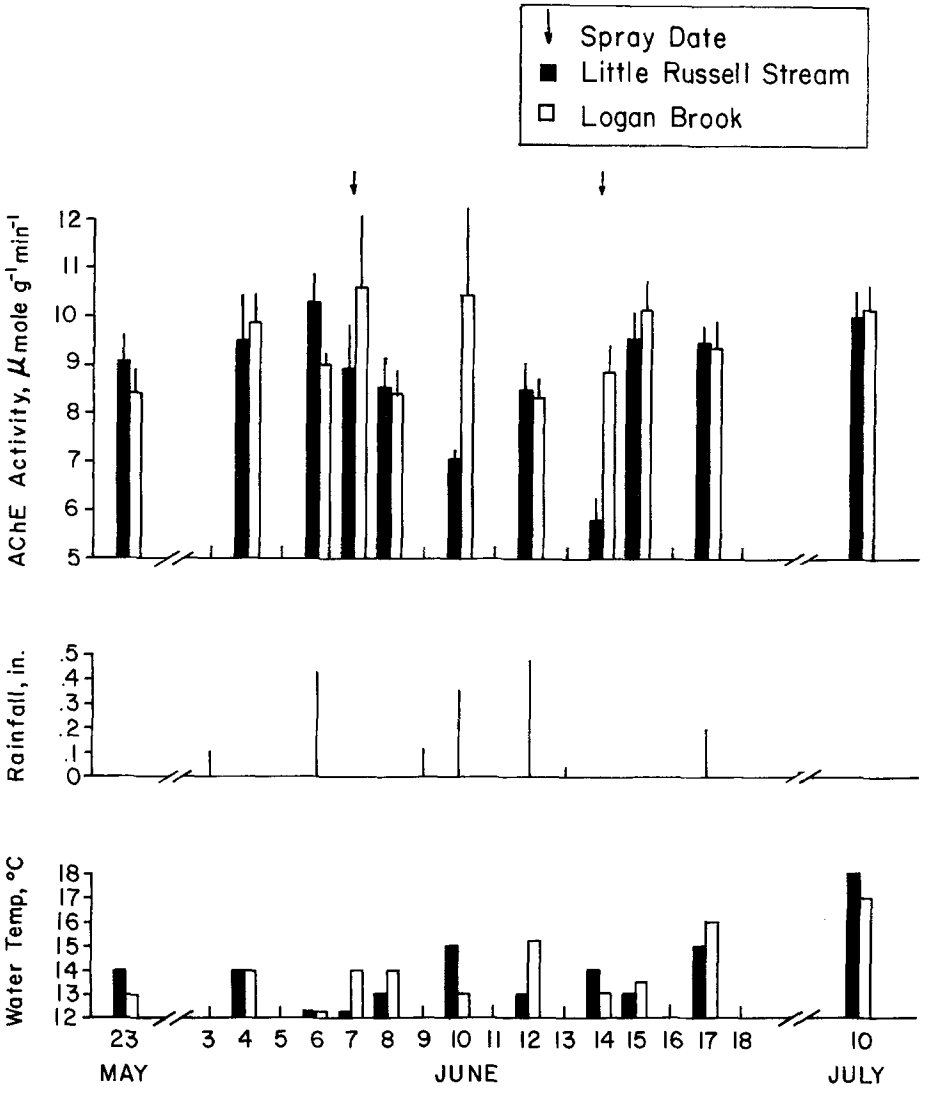

Figure 2. Brain AChE activity (mean ± 1 S E), in brook trout from Little Russell Stream and Logan Brook, rainfall, and stream water temperature.

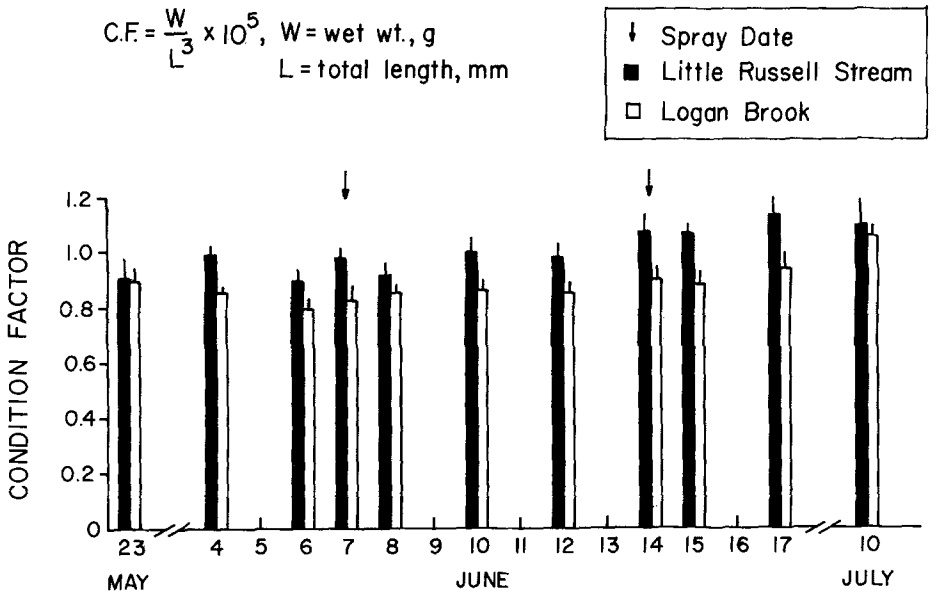

Figure 3. Brook trout condition factor, CF (mean ± 1 S E).

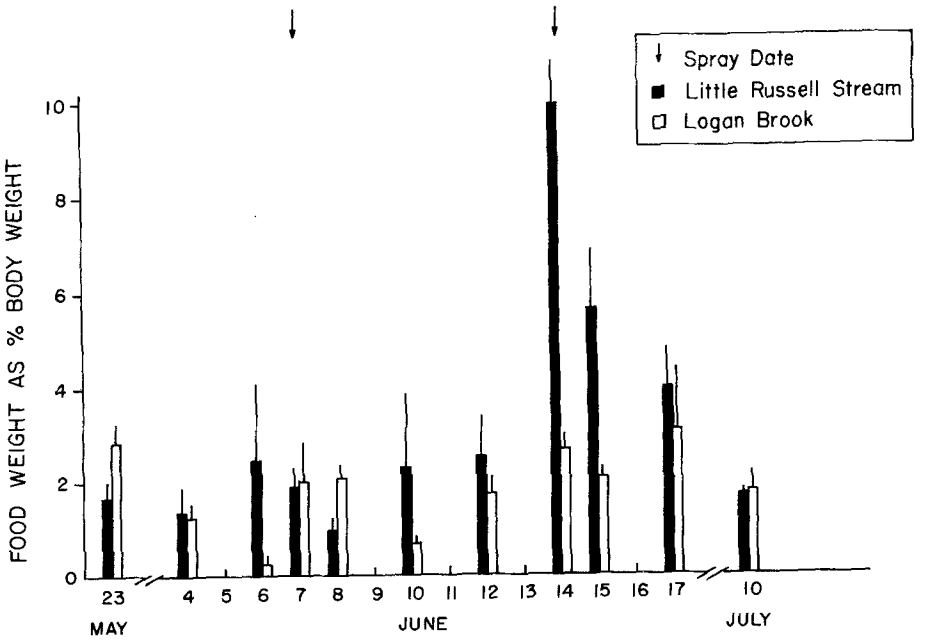

Figure 4. Brook trout stomach contents, expressed as percent of body weight (mean ± 1 S E).

Table 1. Mean numbers of invertebrates (± S E in parentheses) found in brook trout stomachs from Little Russell Stream and Logan Brook, the control stream.

	Coleoptera		Diptera		Ephemeroptera		Trichoptera		Terrestrial	
Date	Little Russell	Logan	Little Russell	Logan	Little Russell	Logan	Little Russell	Logan	Little Russell	Logan
May 23	0.8 (0.4)	2.4 (0.8)	2.6 (2.1)	51.8 (25.6)	0.8 (0.6)	1.2 (0.8)	0.8 (0.4)	2.8 (0.4)	2.4 (1.5)	2.2 (2.2)
June 4	1.0 (0.4)	0	0	0.2 (0.2)	0	0	0.8 (0.5)	0.3 (0.2)	1.2 (0.6)	2.2 (0.9)
June 6	0.4 (0.4)	0.2 (0.2)	0	0	0	0	0.2 (0.2)	0	0.2 (0.2)	0
June 7*	0.6 (0.2)	0.6 (0.4)	83.2 (41.6)	0.2 (0.2)	17.6 (7.2)	0	8.2 (6.7)	1.4 (0.4)	0	0
June 8	0.2 (0.2)	1.4 (0.9)	1.0 (1.0)	0.6 (0.4)	0.6 (0.2)	0.6 (0.4)	1.2 (0.5)	1.4 (0.4)	0.4 (0.2)	3.4 (2.9)
June 10	0.2 (0.2)	0.4 (0.4)	0.2 (0.2)	0.2 (0.2)	2.8 (2.8)	1.2 (1.0)	1.4 (0.6)	1.6 (0.6)	0	0.4 (0.4)
June 12	0.2 (0.2)	0.6 (0.4)	1.0 (0.6)	2.6 (2.4)	0.5 (0.3)	0	1.3 (0.3)	1.8 (0.6)	0	0.2 (0.2)
June 14*	0.4 (0.4)	3.2 (1.2)	4.6 (2.1)	0.2 (0.2)	116.8 (41.5)	0	170.4 (56.9)	1.8 (0.6)	7.6 (3.1)	1.2 (1.0)
June 15	0.6 (0.2)	1.0 (0.6)	2.2 (1.2)	2.6 (1.0)	44.6 (14.8)	0.6 (0.4)	67.8 (18.3)	4.2 (1.6)	3.6 (1.7)	0.2 (0.2)
June 17	1.6 (0.9)	2.2 (1.0)	0	0.2 (0.2)	17.2 (8.6)	0	34.8 (13.5)	2.6 (1.1)	2.4 (1.9)	0
July 10	1.4 (0.2)	0	1.4 (0.8)	3.4 (1.4)	0.6 (0.4)	0.2 (0.2)	2.6 (0.9)	0.8 (0.5)	0	0

*Date of spraying

relatively unchanged for 8 days after application, and MARANCIK (1976) found that the depression persisted for at least 48 h post-spray. Possibly AChE activity recovery occurs more quickly after fish are exposed to the lower application rate, even though the magnitude of the depression is similar to that caused by the higher rate.

The impact on brook trout of AChE depression of 15 to 34% persisting for 24 h or less is unknown. COPPAGE (1972) reported that exposure to several organosphosphates resulted in mortality of fish only when AChE was inhibited 82% or more, and WEISS (1958) found that mortality did not occur at AChE inhibitions of less than 50%. POST & LEASURE (1974) showed that 32% inhibition of AChE activity in brook trout caused a 16% decline in an index of activity. The short duration of inhibition in the present study suggests that adverse effects were unlikely.

Condition factor was consistently higher in Little Russell Stream fish and may reflect the presence of more abundant food in this stream. There was no indication that pesticide application in this study caused sufficient stress, or reduced food abundance sufficiently to affect condition factor.

The amount of food in a fish, expressed as percent of body weight, did not change after the first spraying but increased after the second. The increase in food weight per fish in Little Russell Stream on June 6, before the first spraying, is unexplained. The increase after the spraying resulted primarily from increased numbers of Ephemeroptera and Trichoptera, and to a lesser extent Diptera. Plecoptera, known to be sensitive to application of carbaryl (TRIAL 1978) were rarely found in the stomachs. Terrestrial insects in the diet increased only slightly after the spraying.

Dead or moribund fish were not observed in the treated stream, and the bioassay with young-of-the-year brook trout indicated no mortality. The single mortality observed after the first spraying, as well as the two preceding the spraying, probably were a result of handling stress or other causes, rather than pesticide toxicity.

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