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Densities of Mink Frogs, *Rana septentrionalis,* in New Brunswick Forest Ponds Sprayed with the Insecticide Fenitrothion

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From 1976 through 1993 up to 3.6 million hectares of New Brunswick forest were sprayed annually with the broad-spectrum insecticide fenitrothion (0,0-dimethyl 0-(4-nitro-m-tolyl)phosphorothioate) to minimize spruce budworm (Choristoneura fumiferana) damage to softwood (Kuhnke 1989). Although fenitrothion has a half-life in stream water that is estimated to be only 6-10 hr, the environmental impact of some forestry uses of this pesticide has been deemed unacceptable in Canada (Pauli et al. 1993). Registration for broad scale application of fenitrothion for control of spruce budworm and hemlock looper (Lambdina fiscellaria) will be phased out by the end of 1998, while other minor uses will still be permitted (IECPM 1995).

Until 1993 there were no pesticide regulations preventing the incidental spraying of forest ponds of < 40 ha, habitat important to amphibians. Although the direct or indirect effects of pesticides may have contributed to world-wide declines of amphibians, there is little field evidence to support this (Bishop 1992). Here we provide preliminary evidence that supports such a link involving fenitrothion and the mink frog (Rana septentrionalis). Abundance of aquatic vegetation and water quality are also examined and their association with frog abundance evaluated. Our objective is to assess whether lower frog populations were present in forest ponds repeatedly sprayed with fenitrothion over several years when compared to ponds that had received little or no exposure to fenitrothion during the same period.

MATERIALS AND METHODS

Nine study ponds/lakes in northern New Brunswick were selected and their fenitrothion spray histories over the period 1987-90 were ranked as low, medium, and high. The region is not pond or lake dense and the number of study sites was limited. The low fenitrothion spray zone had

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not been sprayed in 1987-90, the medium spray zone had been sprayed only in 1990, and the high spray zone had been sprayed in three or four of the previous four years. Sprayed forest had received a dose rate of two applications of 210 g active ingredient•ha^{-1} 5-15 days apart during May and June. The sites selected are all permanent water bodies of 1-22 ha, are at elevations of 244-1550 m and are all in deciduous forest within 35 minutes latitude of each other. Ponds were transect censused for frogs in the third weeks of June, July and August 1991 in the manner described in McAlpine (1997) by individuals with no knowledge of the spray history of the ponds. Mink frogs were the only frog abundant enough to permit comparative analyses. Water temperature and pH were recorded and water samples collected monthly and analyzed for the following by the Water Quality Branch, Environment Canada: specific conductance, turbidity, colour, alkalinity, organic carbon, inorganic carbon, calcium, magnesium, potassium, sodium, sulphate, silica, nitrogen, nitrate, extractable manganese, extractable aluminum, extractable iron, and total phosphorus. Abundances of emergent vegetation and the submerged vegetation mat were estimated and ranked as low (<l0% cover), medium (l0-50% cover), or high (>50% cover) for each transect.

Mean frog densities were calculated for each month by site and transformed using natural logarithms (1n x + 1) to meet the assumptions of normality and homogeneity of variances. A nested ANOVA was used to compare frog densities among spray zones, ponds, and months (ponds were nested within spray zones). To overcome the inequality in the number of transect counts completed between ponds, and between months in the same ponds, least-squares means (LS means) were used to determine unbiased mean densities for each site. These 1n-transformed means (and SE) were back-transformed to give geometric means of the raw data. LS means of the 1n-transformed frog densities were also calculated for each of the three spray zones and Bonferroni comparisons were used to test for differences in frog densities among the zones. Multiple regression was used to examine the associations among frog abundance, fenitrothion spray history, and the habitat parameters. Water quality data, sampling month, and ranks for vegetation abundance and spray zone, were incorporated into a stepwise multiple regression model to evaluate degree of association with the In-transformed frog densities (SYSTAT 1992).

RESULTS AND DISCUSSION

The 1n-transformed mink frog densities were significantly different among spray zones (nested ANOVA , $F_{[2,93]}=38.9$, $p < 0.001$) and among ponds within the spray zones ($F_{[6,93]}=7.8$, $p < 0.001$). There were no consistent differences among the monthly frog counts in the ponds ($F_{[2,93]}$=0.11, p=0.9). When the data were combined within spray zones, the LS mean (± SE) ln-transformed frog density of the high spray sites (1.4 ± 0.1/100 m^2) was significantly lower than means for either the low spray (2.6 ± 0.2) sites or the medium spray sites (3.4 ± 0.2), as judged by paired comparisons ($p < 0.001$). Additionally, the low spray sites had a lower mean frog density than the medium spray sites (p=0.01). Ponds that were sprayed with fenitrothion in repeated years (high spray) all had lower mean frog densities than the low or medium spray ponds (Table 1). Also, although the mean counts in the high spray ponds were very similar, the counts in the two medium spray ponds were very different. Multiple regression of all 20 water quality parameters, the vegetation ranks, the spray zone ranks and the sampling month accounted for 53% of the observed variation in the transformed frog densities (r^2=0.53, $F_{[3,100]}$=37.2, $p < 0.001$). Three of the independent variables were significantly associated with frog densities. In decreasing order of importance these were; spray zone (b'=-0.56, t=-5.1, d.f.=100, $p < 0.001$), submergent vegetation (b'=0.48, t=6.4, $p < 0.001$), and sulphate concentration (b'=-0.32, t=-3.0, p=0.004). Thus there tended to be more mink frogs in ponds that had been sprayed less often with fenitrothion, that had more submergent vegetation, and that had lower sulphate concentrations.

Table 1. Least-square geometric means for mink frog densities in New Brunswick forest ponds. Back-transformed from ln-transformed data (ln x + 1).

Site	Spray zone	Geometric mean (frogs/100^2) $\bar{x}$	Lower SE	Upper SE
Black Lake	low	7.09	5.27	9.45
South Lake	low	20.95	15.81	27.67
Berry Brook Pond	medium	65.43	49.16	86.97
Juniper Lake	medium	12.05	9.21	15.69
Camel Back Lake	high	3.86	2.64	5.50
Forty Mile Pond	high	3.52	2.38	5.04
Forty Mile Lake	high	3.99	2.73	5.67
Indian Lake	high	6.15	4.52	8.26
McCormack Lake	high	0.71	0.37	1.14

It is not surprising that greater mink frog abundance was correlated with increasing amounts of aquatic vegetation, which provides cover for adult and larval mink frogs. Structurally complex habitats can reduce encounter rates between prey and predator by providing cover for the former and creating deterrents to foraging by the latter, thus increasing prey survival rates (Babbitt and Jordan 1996). More heavily vegetated ponds may also provide a greater abundance of prey (Campeau et al. 1994).

Higher sulphate levels in pond water were associated with fewer mink frogs. Increased sulphate levels may result from inputs of acidic precipitation from distant or local SO_2 sources, or leaching of natural gypsum deposits. In their study of Nova Scotia amphibians, Dale et al. (1985) found mink frogs in only 10 of 78 lakes and ponds surveyed. Those 10 water bodies all had lower than average sulphate concentrations. Of the 8 anurans in the Nova Scotia survey, mink frogs occupied waters with the lowest and narrowest range of sulphate concentrations, suggesting that the species may be sensitive in this respect.

Although field studies in New Brunswick associated with the aerial application of fenitrothion have found no acutely toxic effects on amphibians (Pearce and Price 1977), sublethal impacts such as abnormal swimming behaviour, paralysis, reduced growth, and retarded development, are seen in larval amphibians exposed to 0.2-5.5 mg/L fenitrothion in lab studies (Berrill et al. 1994). Maximum fenitrothion concentrations observed in lentic waters in New Brunswick a few hours after spraying average 0.2 mg/L (Fairchild et al. 1989) with peak concentrations up to 2.5 mg/L in the surface layer of small ponds (Ernst et al. 1991).

Fenitrothion concentrations observed in small forest ponds after aerial spraying are variable, but may be high enough to have sublethal impacts on tadpoles. The surface water concentrations of fenitrothion measured in New Brunswick forest ponds immediately after spraying by Ernst et al. (1991) were in the same range as those which produced temporary paralysis in laboratory tests with leopard frog (R. pipiens) and green frog (R. clamitans) tadpoles, and irreversible paralysis in bullfrog (R. catesbeiana) tadpoles (Berrill et al. 1994). Although field surveys in New Brunswick have found no evidence of acute lethal effects on amphibians, no intensive field studies have tried to document sublethal effects on amphibians. Such effects as paralysis, reduced growth or delayed metamorphosis of tadpoles may decrease their survival by making them more vulnerable to predators or prolonging their vulnerability over a longer period of time.

The indirect effects that fenitrothion may have on forest communities have not been extensively studied. Millikin and Smith (1990) recorded significant decreases in the arthropod food of songbirds and changes in foraging behaviour of some avian species following an experimental application of fenitrothion. Reductions in seed and fruit production have been documented for forest plants whose insect pollinators were reduced by fenitrothion spraying (Plowright 1977). Symons (1977) calculated a potential reduction of 15% in annual growth of Atlantic salmon if insect biomass in streams was reduced by fenitrothion by > 65%. Kent and Caux (1995) report significant growth inhibition in phytoplankton exposed to fenitrothion concentrations of 1.0 mg/L or above.

Fenitrothion may reduce the amount of invertebrate prey available to frogs. Total benthic arthropod densities decreased about 50% in bog ponds which had surface water concentrations of 42-81 ug/L fenitrothion after an experimental fenitrothion application (Fairchild and Eidt 1993). Recovery of some invertebrate families took more than 12 months. Eidt (1981) found a significant reduction in arthropod biomass but complete recovery within 50 days after fenitrothion was experimentally applied (73 ug/L) in a New Brunswick stream. The impacts observed by Fairchild and Eidt (1993) were associated with fenitrothion concentrations two orders of magnitude lower than the maximum recorded by Ernst et al. (1991) after current operational spraying. Thus, there is ample evidence that dosages of fenitrothion in use in New Brunswick could produce a wide-spectrum kill of arthropods that are prey of amphibians inhabiting forest ponds. Even the relatively short-term effects reported by Eidt (1981) could be significant given the relatively short feeding season for amphibians at Canadian latitudes.

Our results show low mink frog numbers in all forest ponds receiving repeated applications of fenitrothion (see McAlpine (1997) for untransformed monthly estimates from individual ponds). This is consistent with an hypothesized indirect impact of fenitrothion on frog abundance through reductions in the invertebrate prey base or sublethal effects leading to increased mortality of tadpoles.

That the regression model accounted for about half of the variation in frog numbers among ponds suggests that other, unmeasured factors are also influencing mink frog abundance. Some possible influences which were not measured in this study were the abundance of predators, the level of human disturbance, the potential for immigration or recolonization of the ponds, or other unmeasured pollutants or stresses. We suggest that some of these unmeasured factors may have been important in determining frog numbers in low spray ponds, where we recorded fewer frogs overall than at medium spray sites. We emphasize that none of the associations reported here demonstrate cause and effect and that frog populations are known to fluctuate greatly, sometimes on an annual basis (Pechmann et al. 1991).

Although our results support the hypothesis that fenitrothion may adversely affect mink frog populations in forest ponds sprayed repeatedly, our sample numbers of ponds are small. Controlled experiments and more extensive field surveys of several seasons duration will be necessary to confirm these results and prove causal mechanisms.

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