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# Effects of Naled, Synergized, and Non-Synergized Resmethrin on the Swimming Performance of Young Trout

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Over the last 30 years, townships in the Adirondack Mountains of New York State (NYS) have used numerous insecticides to control nuisance black fly and mosquito populations. In recent years towns have conducted aerial spray programs that use naled and resmethrin as adulticides (Dean and Ford 1983; Ford 1990). The accidental spraying of small headwater streams in the Adirondack Mountain region is likely to produce short, pulse type exposures of insecticides to fish (Norris et al. 1983). While lethal concentrations might not exist, the swimming ability of young trout in these streams may be impacted in such a way as to reduce the survival of these fish (Colquhoun et al. 1984; Paul and Simonin 1995).

This study compares the effects of two commercial formulations of these adulticides (Dibrom and Scourge) commonly used in NYS. We tested the swimming performance of young brook trout (*Salvelinus fontinalis*) following short term exposures (6 hr) to these adulticides. Dibrom contains the active ingredient naled, which is an organophosphate insecticide. Scourge contains the synthetic pyrethroid resmethrin and the synergist piperonyl butoxide (PBO) which may exacerbate the toxicity of resmethrin to non-target organisms. In order to account for any impact of the synergist we compared Scourge with a non-synergized formulation of resmethrin (SBP-1382).

## MATERIALS AND METHODS

The naled used in this study was Dibrom Concentrate (Valent USA Corp., Walnut Creek, CA) which is 85% w/w naled. The two resmethrin formulations used were produced by Roussel Uclaf Corp. (Englewood Cliffs, NJ). Scourge 4% + 12% MF is a PBO synergized formulation of resmethrin which contains 4.14 % w/w resmethrin and 12.42% w/w PBO. SBP-1382 Insecticide 4.22 MF is a non-synergized formulation of resmethrin which contains 4.22% w/w resmethrin.

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The fish used in our studies were Rome Lab strain domestic brook trout obtained from the NYS Rome Fish Hatchery. These fish were raised by the NYS Aquatic Toxicant Research Unit in Rome, NY. The age of brook trout used was 30-42 d post feeding (mean total length = 35.7 mm, range = 29-40 mm). The trout were not fed 24 hr prior to or during the tests.

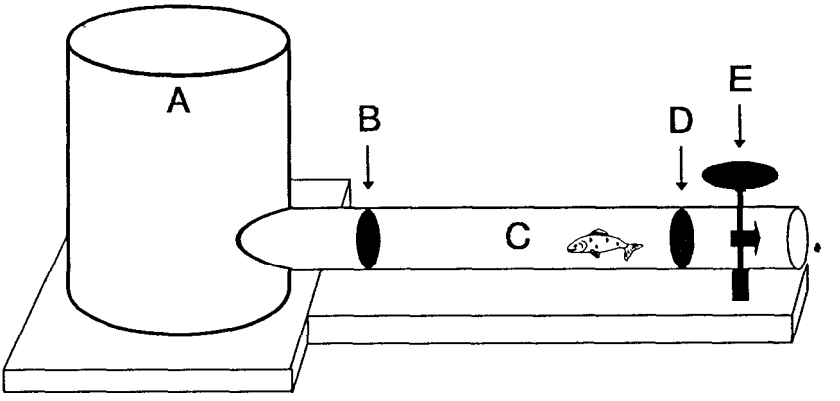
Trout were exposed to the pesticides in 2-L glass containers with 1.5 L of test solution. Rome spring water was used as the dilution water (pH = 7.61, hardness = 132 mg/L $CaCO_3$, total alkalinity = 114 mg/L $CaCO_3$, conductivity = 305 µmho/cm). Temperature was held at 9.0 ± 0.5°C using a thermostatically controlled water bath. Dissolved oxygen was ≥ 9.2 mg/L.

Fresh stock solutions of each formulation were prepared each day by dissolving the insecticides in acetone to make a 168,000 µg/L naled or 10,000 µg/L resmethrin solution. These stock solutions were used to mix the exposure solutions. Test concentrations for naled were 24 and 46 µg/L during the first week of tests and 91 µg/L during the following week. Test concentrations used for both resmethrin formulations were 1.6 and 3.2 µg/L during week one and 6.3 µg/L during week two. Control containers were mixed with a volume of acetone equal to that of the highest test concentration. In order to allow a direct comparison of the potential impact of the two different active ingredients, the concentrations tested were proportional to the maximum application rates (112 g naled/ha; 7.84 g resmethrimha). In this way 24 µg/L naled is equivalent to 1.6 µg/L resmethrin, 46 µg/L naled is equivalent to 3.2 µg/L resmethrin, and 91 µg/L naled is equivalent to 6.3 µg/L resmethrin.

Each exposure solution was prepared 20 min prior to adding trout. Each pesticide exposure solution was mixed in pairs to permit simultaneous testing in each of two stamina chambers. A pair of young trout were placed into the pair of solutions and held for 6 hr in a constant temperature water bath. A new pair of test solutions was started every 20 min to allow time to test the two fish, one in each chamber.

A simple non-recirculating stamina chamber was used to test the trout (Figure 1). A head chamber supplied water at a constant pressure. The head chamber was continuously filled to overflowing during testing. The test chamber portion of the apparatus was made of clear acrylic tubing (length = 61 cm, interior diameter = 4.5 cm). A set of 3 screens located immediately upstream of the test chamber was used to confine the fish and produce laminar flow (Colquhoun et al. 1984). The downstream screens also confined the fish and were removable so fish could be placed in the stamina chamber. The velocity of the water through the stamina chamber was controlled by incremental setting of a gate valve located on the lower end of the apparatus. Two chambers were utilized to facilitate testing. Each chamber was calibrated by determining the time required to fill a known volume container at various settings of the gate valve. The velocity was calculated by the equation:

$$V = F/A$$




A: Head chamber for constant pressure. Height = 30 cm Interior diameter = 12.5 cm
B: Front union with screen barrier to confine fish and produce laminar flow.
C: Test chamber of clear acrylic tubing. Length = 61 cm Interior diameter = 4.5 cm
D: Rear union with screen barrier (removable to insert fish).
E: Calibrated gate valve for velocity control.

**Figure 1.** Non-recirculating stamina chamber (not to scale).

where: V = velocity (cm/s)
F = flow (ml/s)
A = cross-sectional area of the chamber (15.90 $cm^2$)

Three measurements were taken at each valve setting and regression analysis was used to produce the following equations.

Test Chamber A: $V = -6.79 \pm 6.89 (S)$ $r^2 = 0.990$ $(p < .0001)$

Test Chamber B: $V = -3.59 \pm 7.52 (S)$ $r^2 = 0.998$ $(p < .0001)$

where: V = velocity (cm/s)
S = valve Setting (complete turns)

A lack of linearity in the velocity response was observed for each chamber with valve settings less than 1 full turn and for the increment of the last full turn on each valve. We, therefore, did not include these settings in the regression analysis. No valve settings at either of these extremes were used during testing. We used the regression equations to select valve settings which would produce the desired velocities in each chamber.

We compared the valve settings obtained with our regression equations with those obtained by Colquhoun et al. (1984), when these test chambers were used in the late

1970s. Most of the valve settings were identical, and no setting varied by more than 1/8 of a turn.

Testing was conducted on two consecutive weeks. During the first week the lowest two concentrations of each insecticide were tested along with an equal number of control fish. All of these tests were conducted over a 3-d period. During the second week the highest concentrations of each insecticide were tested, also with an appropriate set of controls. All of these tests were completed over a 2-d period. We stratified our testing by each day of the week (testing equal numbers of fish in each pesticide-concentration combination) in case significant growth occurred during the 9 days of testing.

Following the 6-hr exposure to either control or pesticide solutions the trout were placed into the stamina chambers. We finished filling the chambers slowly, so as not to disturb the trout. The procedure used was identical to that of Colquhoun et al. (1984). The flow through each of the stamina chambers was adjusted to produce velocities of 7.0, 11.1, 14.8, 18.6, and 22.4 cm/s. The velocity was held at each level for 1 min before increasing to the next level. The time to exhaustion was defined to be the total time from first exposure to the lowest velocity until impingement on the rear screen. Trout which could maintain their position for 10 min were recorded as > 600 s. The velocities selected for our testing are well within the range encountered by trout in mountain streams during normal flows (Hynes 1970). A total of 12 trout were individually exposed and tested in the stamina chambers for each pesticide concentration.

We used nonparametric statistics (Mann Whitney test) to compare the effects of the various pesticide-concentration combinations on swimming time because several of the fish were able to swim > 600 s. Nonparametric statistics allowed the utilization of these extreme swimming times. We also examined the effect of length on swimming performance in the control fish using regression analysis.

## RESULTS AND DISCUSSION

We detected a statistically significant ($p < 0.001$) increase in length of the test fish between the 2 weeks of testing; therefore we did not pool the results of the stamina tests between the 2 weeks. We found no statistically significant differences ($p > 0.50$) in length between any of the pesticide-concentration combinations including the controls within each of the weeks. This allowed us to pool the data within each week. Examining the controls only, we were able to determine a significant relationship between length and swimming performance. This relationship is not surprising and has been discussed by other researchers (Brett and Glass 1973, Jones et al. 1974, Moyle and Cech 1982). This result stresses the importance that stamina studies conducted on young, rapidly growing fish need to be conducted over very short time periods.

**Table 1.** Median swimming times (and ranges) for brook trout (mean length = 34.6 mm) exposed to naled, synergized and non-synergized resmethrin tested during week one.

| Pesticide | Concentration (µg/L A.I.) | Median Swim Time (s) (range) |
|---|---|---|
| Control | 0 | 255 (52 - > 600) |
| Naled | 23 | 280 (124 - > 600) |
| | 46 | 276 (16 - > 600; |
| Synergized Resmethrin | 1.6 | 290 (152 - 401) |
| | 3.2 | 130 (75 - 225)* |
| Non-synergized Resmethrin | 1.6 | 265 (150 - 467) |
| | 3.2 | 182 (78 - 376) |

* Indicates a swimming time which is significantly ($p < 0.01$) lower than that of the control

Swimming performance of fish exposed for 6 hrs to the PBO synergized resmethrin was impaired at a lower concentration than that of the other pesticides tested (Tables 1 and 2). Swimming stamina of brook trout decreased significantly ($p < 0.01$) when exposed to synergized resmethrin at 3.2 µg/L. The non-synergized resmethrin only reduced swimming stamina ($p< 0.0001$) at the highest level tested (6.3 µg/L). In addition, there was a significant difference ($p < 0.01$) in swimming stamina between the two resmethrin formulations at 6.3 µg/L. Fish exposed to the synergized resmethrin had significantly shorter swimming times. In toxicity studies of non-synergized resmethrin, Marking and Bills (1984) noted changes in swimming behavior of green sunfish (*Lepomis cyanellus*), yellow perch (*Perca flavescens*), and carp (*Cyprinus carpio*) following 6-hr exposures to resmethrin at 100 µg/L. These changes included erratic and inverted swimming and in some cases swimming stopped altogether.

**Table 2.** Median swimming times (and ranges) for brook trout (mean length = 37.6 mm) exposed to naled, synergized and non-synergized resmethrin tested during week two.

| Pesticide | Concentration (µg/L A.1.) | Median Swim Time (s) (range) |
|---|---|---|
| Control | 0 | 527 (246 - > 600) |
| Naled | 91 | 426 (65 - > 600) |
| Synergized Resmethrin | 6.3 | 11 (6 - 25)* |
| Non-synergized Resmethrin | 6.3 | 27 (10 - 188)* |

Indicates swimming times which are significantly ($p < 0.0001$) lower than that of the control. In addition, these two swimming times are significantly different ($p <0.01$) from each other.

While the 100 µg/L concentration is 20 times greater than those used in our study, the effects on swimming ability are consistent with our study.

Swimming stamina did not decrease significantly at any of the tested naled concentrations. Colquhoun et al. (1984) tested the stamina of brown trout (*Salmo trutta*) (mean total length = 27.6 mm) exposed to naled at 84 µg/L for 24 hr prior to testing. The stamina of these brown trout was reduced by 57% following the longer exposure time and the same stamina test procedure. Maki et al. (1973) report that golden shiners *(Notemigonus crysoleucas*) exposed to 5.0 mg/L naled for 0.3-4.0 hr were very lethargic, rarely attempting to swim against the current. Mayer and Ellersieck (1986) report 24-hr and 96-hr LC50s to lake trout (*Salvelinus namaycush*) of 113 µg/L and 87 µg/L, respectively. Apparently, naled concentrations higher than those we tested, or longer exposure times would be necessary to cause a decrease in brook trout swimming stamina.

We made direct comparisons between the three pesticides since the concentrations selected for testing were related to each other based upon their maximum labelled application rates. Resmethrin, in either formulation, had a greater effect on trout stamina than naled. The synergist greatly increased the effect of resmethrin on brook trout stamina, following the 6-hr exposures. We reported (Paul and Simonin 1995) 24-hr and 96-hr LC50s for synergized resmethrin to brook trout of 3.07 µg/L and 1.83 µg/L, respectively. We also reported 24-hr and 96-hr LC50s for non-synergized resmethrin of 3.26 µg/L and 1.69 µg/L, respectively. While these LC50s indicate no difference in toxicity between the two formulations of resmethrin, 6-hr exposure tests conducted at that time demonstrated a large difference in the toxicity. The current stamina study confirms these earlier observations. The synergist PBO greatly enhances the toxic effects of resmethrin during short exposures.

Several factors control the length of time and concentration to which stream fish might be exposed to resmethrin or naled. The length of a stream section sprayed, coupled with its flow, determines the amount of time which fish will be exposed. The depth of a stream, along with the physical/chemical properties and application rate of the pesticide, determine the concentration in a stream. Both pesticides are short-lived. Both are degraded by sunlight, but mosquito and blackfly control operations commonly take place in the evening (Dean and Ford 1983, Ford 1990). The potential for chronic toxicity in streams is very small; therefore concern is primarily directed toward the impacts of short pulses of these pesticides. Colquhoun et al. (1984) present a scenario of a mountain stream 0.2 m deep being accidentally sprayed by naled. They found that brown trout could be exposed to levels of naled which might affect swimming stamina. The 24-hr exposure used by Colquhoun et al. (1984) may be too long. A 6-hr exposure would require several km of a mountain stream to be sprayed. While it is unlikely that a section of this length would be accidently sprayed, runoff from an upstream swamp or beaver pond could serve as a source of pesticide to a stream and prolong the exposure. Our 6-hr exposure model should, therefore, be protective enough for most mountain streams. The highest naled concentration which we tested (91 µg/L) is slightly greater than that of Colquhoun et

al. (1984) and did not have a significant effect on swimming stamina following the 6-hr exposure. If we applied the same 0.2 m deep mountain stream senario to the accidental spraying of resmethrin (either formulation), the resulting application would produce a concentration of 3.9 mg/L. This resmethrin concentration is very close to the tested concentration of 3.7 µg/L. We predict that following a 6-hr exposure the synergized resmethrin would have a significant effect on the swimming performance of young brook trout while the non-synergized formulation would not. Consequently the effect of the synergist should be considered when making decisions regarding the possible impact of synergized resmethrin on non-target aquatic animals.

Changes in swimming performance can have effects on long term survival (Woodward et al. 1987). Stream dwelling fish must be able to maintain themselves against the current. Many stream dwelling fish protect themselves from the direct force of the current by selecting sites in a stream protected by rocks or other obstructions (Hynes 1970). Yet even these fish need to move into the current to feed and escape predators (Bachman 1984). Fish and other aquatic animals in ponds also need to capture food and avoid predators. Kutka (1994) demonstrated that even small reductions in swimming activity greatly reduced survival of salamander larvae in the presence of predaceous diving beetles. In addition, there is a question of whether fish exposed to contaminants are able to recognize shelter (Colquhoun et al. 1984). We observed that some fish exposed to resmethrin seemed unable to maintain their position near the bottom of the exposure containers. Many fish exposed to resmethrin have been seen gulping air (Marking and Billings 1984, Paul and Simonin 1995, this study). This would force exposed fish to swim against the current for much longer periods of time. The inability to maintain position against the current resulting from reduced swimming stamina will lead to decreased survival of trout.

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