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[Article]

# Rotenone Toxicity to Rainbow Trout and Several Mountain Stream Insects

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*Abstract.*—The piscicide rotenone has been used for over 70 years to eradicate unwanted fish, but controversy exists regarding its impacts on nontarget organisms, particularly aquatic invertebrates. We evaluated the toxicity of synergized Nusyn-Noxfish and nonsynergized CFT Legumine rotenone formulations in 4- and 8-h exposures to rainbow trout *Oncorhynchus mykiss* and six species of mountain stream caddisflies, mayflies, and stoneflies. We then compared these results with historical treatment data and aquatic invertebrate collections surrounding rotenone treatments in the 1990s that were designed to restore Paiute cutthroat trout *O. clarkii seleniris* to the Silver King Creek basin in Alpine County, California. The toxicity of rotenone was greatest to the trout; the synergist piperonyl butoxide appeared to have no effect on the toxicity of rotenone to the trout but did increase the toxicity to the invertebrates. The mean 8-h concentrations (as rotenone) lethal to 50% of the rainbow trout were 5.3 μg/L for CFT Legumine and 6.2 μg/L for Nusyn-Noxfish; the mean values for invertebrates ranged from 34 to 174 μg/L for CFT Legumine and from 13 to 74 μg/L for Nusyn-Noxfish. These findings corresponded to that observed in Silver King Creek, where three annual treatments of 16–23 μg/L for 6–18 h were successful in extirpating rainbow trout hybrids but caused little change in aquatic insect assemblages. To lessen the impacts of rotenone treatment in mountain streams, project planners should (1) use the lowest rotenone concentration and duration needed to accomplish the treatment objective (we suggest 25–50 μg/L for <8 h) and (2) avoid using formulations containing the synergist piperonyl butoxide.

The piscicides rotenone and antimycin are tools often used to restore native trout by enabling eradication of nonnative fishes (McClay 2000, 2005; Finlayson et al. 2002). Although procedures vary with on-site considerations and species targeted for removal, the general approach is to chemically treat for several years a stream reach that is isolated by barriers, either natural or artificial, and subsequently stock the stream with native fish from extant wild or hatchery populations. Stream reaches, lakes, and fish populations are then connected, working downstream with successive chemical treatments. Restored systems may be stocked from donor sources until the population is self-sustaining.

Piscicides can potentially have direct environmental impacts on esthetics, air quality, hydrology and water quality, hazards and hazardous materials, recreation, and biological resources (McClay 2000). Impacts to aquatic invertebrate assemblages are a particular concern. Impacts to invertebrate assemblages from rotenone treatments in natural ecosystems have been studied since the 1960s. Results have varied considerably with exposure concentration and duration, effects being nearly always greater at higher concentrations and longer durations. High concentrations of rotenone (>100 μg/L) and long treatment durations (>8 h) have typically resulted in severe impacts to invertebrate assemblages (Binns 1967; Mangum and Madrigal 1999; Darby et al. 2004) and the immediate and short-term loss of many, if not all, aquatic invertebrates in terms of both taxa and individuals. Conversely, lower rotenone concentrations (<50 μg/L) and shorter treatment durations (<8 h) have resulted in less impact to invertebrate assemblages (Cook and Moore 1969; Maslin et al. 1988; Trumbo et al. 2000a, 2000b; Whelan 2002).

The toxicity of rotenone to fish appears to vary little

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TABLE 1.—Three-, 6-, and 24-h LC50 of rotenone (μg/L) for several species of fish (Marking and Bills 1976) and invertebrates (Chandler and Marking 1982). Toxicity testing was done with Noxfish 5% rotenone. Exposure levels were not confirmed, and values represent lethality at the exposure interval (no further observation period).

| Species | 3 h | 6 h | 24 h |
|---|---|---|---|
| **Fish** | | | |
| Rainbow trout *Oncorhynchus mykiss* | 8.8 | 4.3 | 3.4 |
| Northern pike *Esox lucius* | 9.0 | 2.9 | 2.2 |
| Common carp *Cyprinus carpio* | | 14.0 | 4.2 |
| Channel catfish *Ictalurus punctatus* | 70 | 42 | 20 |
| Bluegill *Lepomis macrochirus* | 21 | 17 | 7.4 |
| Yellow perch *Perca flavescens* | 7.5 | 6.7 | 4.6 |
| **Aquatic invertebrates** | | | |
| Cladocera: *Daphnia pulex* | 4.8 | 1.8 | 1.4 |
| Ostracoda: *Cypridopsis* | 130 | 110 | 24 |
| Odonata: *Macromia* (larvae) | 13,000 | 1,700 | 240 |
| Trichoptera: *Hydropsyche* (larvae) | 400 | 180 | |
| Coleoptera: *Gyrinus* (adult) | 415 | 400 | 180 |
| Gastropoda: *Helisoma* | 1,700 | 1,700 | 1,500 |

among species, the concentrations lethal to 50% of the test animals (LC50s) after 6 h ranging from about 3 to 42 μg/L rotenone (Table 1). Conversely, the toxicity of rotenone to aquatic invertebrates appears to vary widely among species—from 1.8 to 1,700 μg/L rotenone for 6-h LC50 values (Table 1). Ling (2003) and Vinson et al. (in press) reviewed sensitivity data of rotenone and both concluded that, in general, benthic invertebrates appear less sensitive than planktonic invertebrates, smaller invertebrates typically appear more sensitive than their larger counterparts, and aquatic invertebrates that use gills appear more sensitive than those that acquire oxygen cutaneously or through lamellae, or that use respiratory pigments or breath atmospheric oxygen.

A major difficulty associated with accurately assessing past studies of toxicity and impacts of rotenone on invertebrates rests with unverified concentration–response data in the literature. Many studies do not differentiate between rotenone active ingredient and commercial formulation concentrations. The concentration of active rotenone in liquid formulations is normally 2.5% or 5%, and a treatment rate of 1 and 3 mg/L (ppm) formulation (5% active) would yield active rotenone concentrations of 50 and 150 μg/L, respectively. Most lethality levels (96-h LC50 values) in the literature come from tests on formulated products where rotenone exposure levels were typically not verified by chemical analysis (Marking and Bills 1976; Chandler and Marking 1982). Thus, values not verified by analysis may not accurately reflect lethality at the nominal concentration indicated. Rotenone is also detoxified in the liver of fish and possibly in the hepatopancreas of invertebrates by mixed-function oxidase enzymes (Gingerich and Rach 1985; Hollingworth 2001; Ling 2003). Thus, toxicity values from studies where rotenone levels were not confirmed by chemical analysis may not reflect actual exposure rates.

In recent years, rotenone concentrations used in fish removal projects have declined as training of personnel involved with piscicide projects has increased and project objectives have become more focused, so impacts to invertebrate assemblages are likely less than were observed 10 or more years ago (Vinson et al., in press). Nonetheless, the possible negative impacts of rotenone on stream invertebrate assemblages have become a contentious issue (Finlayson et al. 2005). Numerous projects have been delayed or cancelled due to legal wrangling with groups opposed to piscicide treatments (e.g., Californians for Alternatives to Toxic Substances; Center for Biological Diversity; California Regional Water Quality Control Board, Lahontan Region). One such project is a planned rotenone treatment to restore Paiute cutthroat trout *Oncorhynchus clarkii seleniris* to the Silver King Creek basin in Alpine County, California (U.S. Fish and Wildlife Service 2004). This project has undergone numerous delays since 2002 partially due to legal petitions about potential impacts of rotenone to aquatic invertebrate assemblages in Silver King Creek (Williams 2004, 2005; Finlayson et al. 2005).

In order to more accurately assess the potential impact of the proposed rotenone treatment to aquatic invertebrate assemblages in the Silver King Creek basin, we investigated the short-term (4- and 8-h exposures) toxicity of two rotenone formulations to juvenile rainbow trout and several benthic invertebrate species found in the basin. We then relate these results to several years of aquatic invertebrate collections that were made in Silver King Creek surrounding three rotenone treatments conducted between 1991 and 1993.

## Methods

*Laboratory toxicity*.—Invertebrates were collected with kick nets from the East Fork Carson River upstream of Markleeville, Alpine County, California, in September and October 2006 and 2007 for toxicity tests. Invertebrates were sorted from debris in the field, separated into insect orders, placed in plastic bags with oxygenated water, and transported (2-h journey) in chilled ice chests to the California Department of Fish and Game Aquatic Toxicology Laboratory in Elk Grove, California. Upon arrival, the specimens were taken from the plastic bags and placed into 4-L glass containers under static conditions with aeration inside constant temperature (12°C) incubators. The specimens

were further segregated the next day by size and species. Toxicity testing began within 2 to 3 d of collection. Rainbow trout were obtained several days before testing from the California Department of Fish and Game American River Trout Hatchery located in Rancho Cordova, California. The fish were similarly transported to the Aquatic Toxicology Laboratory, kept in 950-L (250-gal) flow-through tanks, and tested within 4 d. Neither fish nor invertebrates were fed during the tests.

Static, 48-h duration toxicity tests on rainbow trout and invertebrates were conducted in moderately hard water of 92 mg/L $CaCO_3$, pH of 7.8, and alkalinity of 62 mg/L $CaCO_3$, in constant temperature (12°C) incubators following standard guidelines (American Society for Testing and Materials 2001). Trout were exposed for 4 or 8 h to five concentrations of rotenone between 2.3 and 39 μg/L derived from nonsynergized CFT Legumine (5% rotenone, C.W.E. Properties Limited, LLC) or synergized Nusyn-Noxfish (2.5% rotenone and 2.5% piperonyl butoxide; Prentiss, Inc.) commercial formulations. Test chambers for trout tests were 2-L or 600-mL Pyrex glass beakers containing 1,200 (Nusyn-Noxfish) or 300 mL (CFT Legumine) of test solution, respectively. There were 10 replicates per treatment group with each replicate having one trout per test chamber. Following rotenone exposures, solutions in the test chambers were exchanged three times with fresh control water to remove all residual rotenone. Test water was also replaced daily with fresh control water. Nusyn-Noxfish was tested with trout having a mean weight of 1.25 g, and CFT Legumine was tested with trout having a mean weight of 0.22 g, resulting in a loading density of 1.04–0.73 g/L, respectively.

Invertebrates were exposed in a similar manner to six concentrations of rotenone between 5.5 and 378 μg/L from CFT Legumine or Nusyn-Noxfish in 48-h tests. Test chambers were 600-mL Pyrex glass beakers containing 300 mL of test solution with one invertebrate per test chamber to minimize cannibalism, maintaining sufficient dissolved oxygen concentration, and to allow for exact identification of all test organisms. There were 5 to 10 replicates per treatment group depending on number of individuals available. Upon completion of the tests or death of individuals, individuals were preserved in ethanol and identified to species. Two species of mayflies (*Rhithrogena morrisoni* and *Baetis tricaudatus* [order Ephemeroptera]), two species of stoneflies (*Oroperla barbara* and *Claassenia sabulosa* [order Plecoptera]), and two species of caddisflies (*Arctopsyche grandis* and *Hydropsyche tana* or *H. ambles* [the two species were indistinguishable] [order Trichoptera]) were tested. Stoneflies were the largest (mean weight = 0.206 g) and the mayflies were the smallest (mean weight = 0.0072 g) invertebrates tested, resulting in a maximum loading density of 0.69 g/L.

Test solutions (the four replicates were drawn from a single test solution) were analyzed for rotenone and rotenolone (major metabolite of rotenone) content using liquid chromatography–mass spectrometry by the California Department of Fish and Game, Water Pollution Control Laboratory, Rancho Cordova. On average, 10% of water samples were spiked with known amounts of rotenone and analyzed; recovery of spiked samples ranged from 74% to 112%. The relative percent difference between duplicate analyses varied from 5.0% to 7.1%, and the minimum reporting level was 2 μg/L. Results were statistically analyzed using the Comprehensive Environmental Toxicity Information System (Tidepool Scientific Software, McKinleyville, California), and reported toxicity (4- and 8-h LC50 values and 95% confidence intervals [CIs]) values are based on measured rotenone concentrations.

The strength of a chemical exposure to an individual species is often expressed as a fraction or proportion of its lethal concentration in relation to that exposure concentration as a toxic unit (TU = treatment concentration/LC50; Rand and Petrocelli 1985). Toxic units were calculated for the species we evaluated in the laboratory based on the mean (11 μg/L) rotenone exposure (6- to 18-h duration) measured in Silver King Creek in 1991–1993 treatments (Trumbo et al. 2000a) using the 8-h LC50 values. If the TU is greater than 1.0, more than one-half of the individuals of that species are killed, and conversely, if the TU is less than 1.0, then less than one-half of those individuals are killed.

*Field study assessment: study site.*—Silver King Creek is tributary to the East Fork Carson River, which drains into the Lahontan basin (Figure 1). The entire Silver King Creek basin occurs within the Carson–Iceberg Wilderness of the Humboldt–Toiyabe National Forest in Alpine County, California. The creek originates at 2,926-m (9,600-ft) elevation and flows north through three distinct valleys for approximately 22.5 kilometers (14 mi), where it meets the East Fork Carson River. Habitat characteristics of Silver King Creek and tributaries were described by the U.S. Fish and Wildlife Service (2004) and Ryan and Nicola (1976).

*Rotenone treatments.*—Rotenone treatments were conducted in Silver King Creek between 1964 and 1993 to remove nonnative rainbow trout which displaced genetically pure Paiute cutthroat trout (Figure 1). Silver King Creek was treated with Pro-Noxfish (2.5% rotenone) in 1964 and 1976, and with Nusyn-Noxfish (2.5% rotenone) in 1991, 1992, and 1993. The

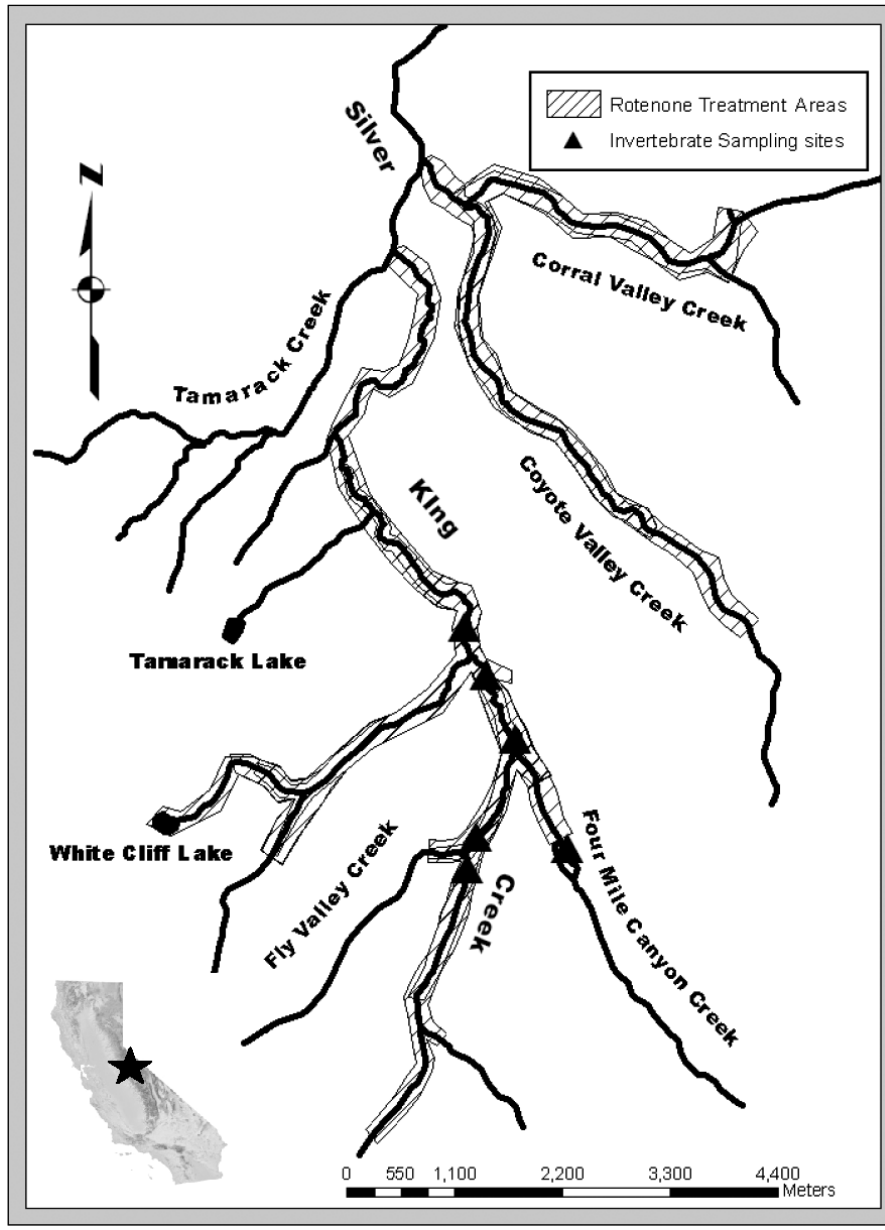


FIGURE 1.—Map of the Silver King Creek basin showing the rotenone treatment reaches and aquatic invertebrate sampling locations. Aquatic macroinvertebrates were sampled between 1991 and 1996.

concentration and duration of rotenone application varied among treatments but generally involved 4–6-h durations at rates of 1 mg/L rotenone formulation (25 μg/L rotenone). In 1991, 1992, and 1993, Nusyn-Noxfish was administered using continuous-flow drip cans (spaced 1–2 h apart [water travel time]), total rotenone exposure lasting from 6 h in upper reaches to 18 h in the lower stream reaches; the discharge of rotenone below the treatment area was neutralized with potassium permanganate (Flint et al. 1998; California Department of Fish and Game 1991, 1992, and 1994). Rotenone concentrations were measured using the methods of Dawson et al. (1983), and maximum concentrations attained were 16–23 μg/L rotenone

(mean concentrations = 10–12 μg/L rotenone; Flint et al. 1998; Trumbo et al. 2000a). Four Mile Canyon Creek was never treated with rotenone.

*Trout mortality assessment*.—In advance of the 1991 chemical treatment of Silver King Creek, approximately 800 juvenile and adult trout were collected by electrofishing and stocked downstream of the project (treatment and neutralization) area to reduce treatment mortality. During and following chemical treatments, fish mortality was assessed by visual surveys, downstream drift into block seines, and by live-cages (Flint et al. 1998). Following chemical treatments, trout population surveys were done annually in Silver King Creek between 1995 and 2008 using electrofishing gear (Flint et al. 1998). To assess if the chemical treatments were successful at removing nonnative rainbow trout, Cordes et al. (2004) collected fin clip samples from 160 Paiute cutthroat trout from five locations in the Silver King Creek drainage during 2000 for genetic analysis. They used nuclear microsatellite and single copy nuclear DNA markers to assess for evidence of Paiute cutthroat trout × rainbow hybridization, which, if present, would suggest that the rotenone treatments were not successful.

*Aquatic invertebrate collections*.—Aquatic invertebrate samples were collected from five locations in Silver King Creek and one location in Four Mile Canyon Creek (Figure 1) between 1991 and 1996. Invertebrate samples were collected once or twice per year during late summer from riffle habitats using a Surber net (0.09-$m^2$, 250-micron mesh net). Three samples were collected per riffle, kept separate, and preserved in a 90% solution of ethanol. Samples were processed by the U.S. Forest Service (USFS) Aquatic Ecosystem Analysis Laboratory, Provo, Utah. Samples were subsampled, and 250–300 invertebrates were removed from each sample and identified. Insects were generally identified to the genus level, Chironomidae were identified to family, and noninsects were generally identified to phylum, class, or order.

*Data analysis*.—Mean differences between aquatic invertebrate samples collected in Silver King Creek (treated) and Four Mile Canyon Creek (untreated) were evaluated for a number of aquatic invertebrate assemblage measures using analysis of variance (ANOVA), with a critical alpha value of 0.05. These measures were selected to be representative of the entire aquatic invertebrate assemblage, a major taxonomic group of aquatic invertebrates (orders Coleoptera, Diptera, Ephemeroptera, Plecoptera, and Trichoptera), and a measure of taxa rarity. For each sampling date and location the following measures were calculated for each composite sample (three Surber samples): total taxa richness (operational taxonomic units [i.e., individuals were identified to a variety of taxonomic levels]; Vinson and Hawkins 1996), total genera richness, genera richness within the dominant orders of aquatic insects, total sample abundance (number per square meter), and the number of taxa with abundances less than 1% of the total assemblage abundance. Data collected from all years, 1991 to 1996, and data collected only treatment years, 1991–1993, were analyzed separately. Results were similar for both data sets, so data from 1991 to 1996 data are presented.

## Results

### *Laboratory Toxicity*

Rainbow trout and invertebrates had dose–response relationships for concentrations of CFT Legumine and Nusyn-Noxfish; control survival was 100% for rainbow trout, 100% for the two stoneflies, 90–100% for mayfly *B. tricaudatus*, 50–100% for mayfly *R. morrisoni*, 40–100% for caddisfly *A. grandis*, and 80–100% for caddisfly *Hydropysche* spp. The lower survival at the end of the 48-h observation period in all three tests with *A. grandis* and one of the four tests with *R. morrisoni* may suggest test conditions less than optimal for these species.

Rainbow trout 4- and 8-h LC50 values were 7.4 and 5.3 μg/L rotenone from CFT Legumine and 7.7 and 6.2 μg/L rotenone from Nusyn-Noxfish (Table 2). Mean invertebrate 4-h LC50 values ranged from 41 to 274 μg/L rotenone from CFT Legumine and 18–96 μg/L rotenone from Nusyn-Noxfish. Mean 8-h LC50 values ranged from 34 to 174 μg/L rotenone from CFT Legumine and 13–74 μg/L rotenone from Nusyn-Noxfish (Table 2). Among invertebrates, LC50 values were highest (most rotenone tolerant) for caddisfly *Hydropsyche* spp., then stoneflies *O. barbara* and *C. sabulosa*, and lowest for mayflies *R. morrisoni* and *B. tricaudatus*.

Rotenone toxicity to trout was greater for the CFT Legumine than the synergized Nusyn-Noxfish formulation (Table 2), suggesting that the synergist piperonyl butoxide had little effect on the toxicity of rotenone for trout. For aquatic insects, the toxicity of the synergized Nusyn-Noxfish was generally greater that the CFT Legumine formulation (Table 2). In theory, the synergist piperonyl butoxide (which is not toxic at the concentration used) increases the toxicity of rotenone by moderating the metabolism (breakdown) of rotenone in the liver (vertebrate) or hepatopancreas (invertebrate), thus allowing for the buildup of toxic levels of rotenone in the organism. It would likely take twice as much Nusyn-Noxfish to yield the effect of CFT Legumine on trout because of the 50% reduction of rotenone in the product, but Nusyn-Noxfish

TABLE 2.—Mean 4- and 8-h LC50 values (μg/L; 95% confidence intervals in parentheses) of rotenone for rainbow trout fry and several species of stream invertebrates from the East Fork Carson River (downstream of Silver King Creek Basin), California. Unless otherwise noted, values represent survival at 48 hours; ND = not determined.

| | 4 h | | 8 h | |
|---|---|---|---|---|
| Species | CFT Legumine | Nusyn-Noxfish | CFT Legumine | Nusyn-Noxfish |
| Rainbow trout | 7.4 (4.8–11.0) | 7.7 (7.6–10.4) | 5.3 (4.2–6.9) | 6.2 (4.5–6.7) |
| Invertebrates | | | | |
| Trichoptera | | | | |
| *Arctopsyche grandis* | ND | 96 (74–117)[a] | 34 (12–55)[a] | 74 (59–88)[a] |
| *Hydropsyche tana/amblis*[b] | 274 (274–ND)[c] | ND | 174 (34–ND) | ND |
| Ephemeroptera | | | | |
| *Baetis tricaudatus* | ND | 18 (11–28) | ND | 23 (11–52) |
| *Rhithrogena morrisoni* | 41 (26–59) | 54 (44–57)[a] | 40 (34–56) | 13 (6.9–17) |
| Plecoptera | | | | |
| *Claassenia sabulosa* | 142 (113–197) | ND | 60 (14–87) | ND |
| *Oroperla barbara* | 197 (77–265) | 70 (57–85) | 102 (67–114) | 57 (52–59) |

[a] 24-h observation.
[b] Species not distinguishable.
[c] 50% survival in highest concentration.

rotenone may be twice as toxic to invertebrates because of the synergist.

Toxic unit analysis suggested that the mean measured rotenone concentration of 11 μg/L used in Silver King Creek treatments between 1991 and 1993 should have resulted in complete mortality to rainbow trout (TU = 1.6–2.4) and some mortality to the two Ephemeroptera spp. (*R. morrisioni* and *B. tricaudatus*) tested (TU = 0.66–1.58 and 0.21–1.0, respectively; Figure 2). The mean measured concentration of rotenone would have resulted in little, if any, mortality to caddisfly *A. grandis* (TU = 0.12–0.19) and the stonefly *O. barbara* (TU = 0.19–0.21; Figure 2).

### *Trout Mortality Assessment*

Following the 1991 treatments, 1,009 adult and juvenile trout and 520 fry carcasses were collected. One hundred seventeen fish carcasses were collected following the second year of treatment in 1992. Of these, 98 fish were from reaches treated in 1992 but not treated in 1991. Since Paiute cutthroat trout exist in headwater tributaries (Fly Valley and Four Mile Canyon) above the project, it was anticipated that some fish would recruit to the project area and succumb to the treatment. Following the third year of treatment in 1993, 23 fish carcasses were collected in or below tributaries with known Paiute cutthroat trout populations. All fish collected visually appeared to be pure Paiute cutthroat trout. One suspect fish was analyzed using allozyme techniques and was found not to contain rainbow trout alleles. All rainbow trout held in live-cages were killed by the chemical treatments (Flint et at. 1998). No trout were captured during an electrofishing survey of the entire Upper Fish Valley during 1994. Fish restocking began from donor stocks in 1994, and the Paiute cutthroat trout population rebounded to historic population levels by 2000. Cordes et al. (2004) concluded from their analysis of fin clips that efforts to eradicate hybridized Paiute cutthroat trout had been successful as no evidence of Paiute cutthroat trout × rainbow trout hybrids were detected from all of the tested populations in the Silver King Creek basin.

### *Aquatic Invertebrate Collections*

Total assemblage abundance was greater at the untreated site (13,944 ± 7,017 [mean ± SD]) than at treated sites (9,637 ± 4,817; $P = 0.0279$). Mean Coleoptera abundance was greater at untreated (2,293 ± 877) than at treated stream sites (865 ± 764; $P < 0.0001$). There were no statistical differences in mean total taxa richness, total genera richness, the genera richness and number of individuals within the major insect orders, and the number of rare taxa between treated and untreated sites (Table 3).

Three genera were collected at the untreated site that were not collected at treated sites: *Ephron* (order Ephemeroptera, family Polymatarcyidae), *Simulium* (order Diptera, family Simuliidae), and *Dolophilodes* (order Trichoptera, family Philopotamidae). Twenty-seven genera were collected at treated sites that were not collected at the untreated site. It should be noted, though, that there were five treated sites and one untreated site, and five times as many samples were collected at treated sites as compared with untreated sites; since, therefore, the likelihood of collecting rare taxa was higher at treated sites, these results should be interpreted with caution. If rotenone was having a strong impact, one might expect that the taxa richness

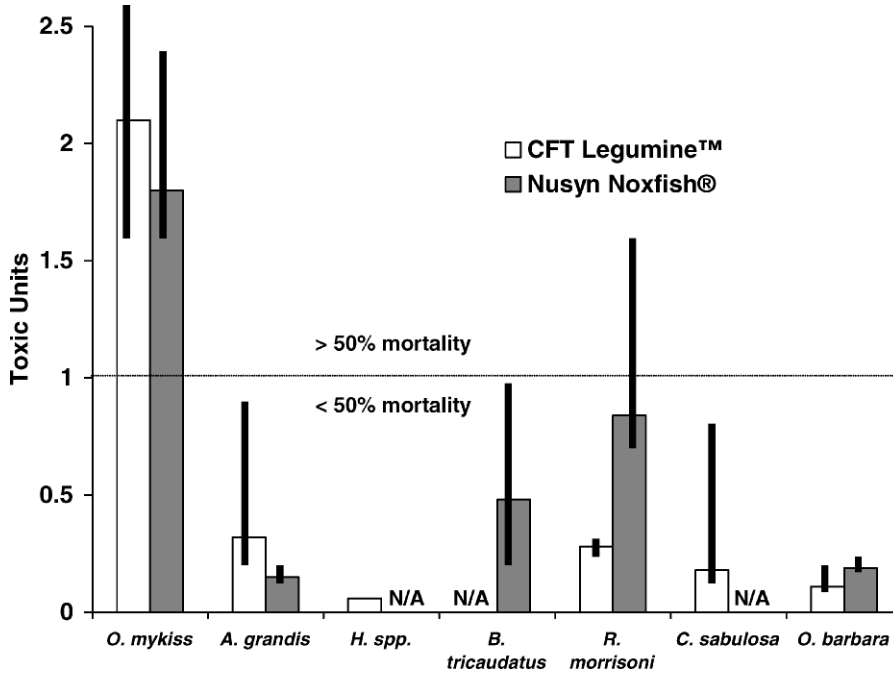


FIGURE 2.—Toxic units (Rand and Petrocelli 1985) for rainbow trout and several species of caddisflies, mayflies, and stoneflies during the treatment of Silver King Creek from 1991 to 1993. The toxic units are the 8-h LC50 values (95% confidence intervals are shown as black lines, where appropriate) from Table 3 associated with the mean rotenone concentration of 11μg/L.

at the untreated site would be greater than observed if the sample size was similar to the treated sites. Several taxa that Engstrom-Heg et al. (1978) observed in laboratory tests to be sensitive to rotenone were collected at treated sites following treatment. These included *Baetis* (order Ephemeroptera, family Baetidae), Perlidae (order Plecoptera), Perlodidae (order Plecoptera), *Rhyacophila* (order Trichoptera, family Rhyacophilidae), and Simuliidae (order Diptera). Taxa with intermediate tolerance to rotenone, as defined by Engstrom-Heg et al. (1978), that were collected at treatment sites included Ephemerellidae and Heptage-

TABLE 3.—Means, SDs, and results of ANOVA to evaluate differences in aquatic invertebrate assemblage measures among rotenone-treated and control stations for samples collected between 1991 and 1996. Abundance measures report the number of individuals per square meter, and richness measures report the number of taxa per three Surber samples. The sample sizes were 45 for rotenone-treated sites in Silver King Creek and 9 for untreated sites in Four Mile Canyon Creek.

| | Control | | Treatment | | | |
|---|---|---|---|---|---|---|
| Measure | Mean | SD | Mean | SD | *F* | *P*[a] |
| Total richness | 35.7 | 4.3 | 34.4 | 5.8 | 0.3667 | 0.5474 |
| Genera | 32.8 | 3.6 | 31.5 | 5.4 | 0.4754 | 0.4936 |
| Total abundance | 13,944.7 | 7,016.9 | 9,637.3 | 4,816.9 | 5.1142 | ***0.0279*** |
| Coleoptera genera | 1.2 | 0.4 | 1.2 | 0.4 | 0.0000 | 1.000 |
| Coleoptera abundance | 2,293.3 | 877.0 | 864.9 | 764.3 | 24.9803 | ***<0.0001*** |
| Diptera genera | 5.2 | 1.2 | 4.8 | 1.0 | 1.1527 | 0.2879 |
| Diptera abundance | 6,044.9 | 3,474.9 | 4,475.7 | 2,815.4 | 2.156 | 0.1480 |
| Ephemeroptera genera | 7.8 | 1.6 | 7.5 | 2.1 | 0.1501 | 0.7000 |
| Ephemeroptera abundance | 3,460.8 | 3,158.7 | 2,646.1 | 1,840.6 | 1.1308 | 0.2925 |
| Plecoptera genera | 3.1 | 1.2 | 2.3 | 1.5 | 2.2493 | 0.1397 |
| Plecoptera abundance | 651.9 | 415.6 | 479.2 | 547.2 | 0.7991 | 0.3755 |
| Trichoptera genera | 3.8 | 1.6 | 3.6 | 1.5 | 0.1078 | 0.7440 |
| Trichoptera abundance | 336.9 | 267.1 | 423.0 | 241.5 | 0.9227 | 0.3412 |
| Rare taxa (< 1% of total abundance) | 22.2 | 4.2 | 19.6 | 4.1 | 3.0229 | 0.0880 |

[a] Bold italics denote significance at $P < 0.05$.

niidae (order Ephemeroptera), Chloroperlidae (order Plecoptera), Limnephilidae (order Trichoptera), and Tipuliidae and Chironomidae (order Diptera).

## Discussion

Our rotenone toxicity test results for rainbow trout and the caddisfly *Hydropsyche* spp. were consistent with those of previous laboratory studies (Table 1). Our results for aquatic invertebrates were also similar with respect to both the variation in toxicity we observed among species and in the toxicity values we observed. Assuming our laboratory results are representative of toxicities for a broader range of mountain stream invertebrate species, the results for Nusyn-Noxfish suggest that a rotenone treatment with a mean rotenone concentration of 11 μg/L with an exposure time of 6–18 h would result in complete mortality to trout and some mortality of invertebrate species, but many invertebrate species would survive. Engstrom-Heg et al. (1978) concluded that there was no level of application at which fish could be eliminated without at least some loss of aquatic invertebrate species; treatments of 10 ppm-hours (e.g., a treatment rate using 1 ppm of 5% rotenone product [50 μg/L] for 10 h) would likely be toxic to rotenone-intolerant species and likely not kill rotenone-tolerant species. This 10 ppm-hours scenario by Engstrom-Heg et al. (1978) would likely result in a 50 μg/L rotenone exposure. This rotenone exposure would have increased the impact on the invertebrates (TU $>$ 1.0) for both species of mayflies *B. tricaudatus* and *R. morrisoni*, and for the caddisfly *A. grandis* in Silver King Creek.

This idea appears supported by our evaluation of the long-term aquatic invertebrate collections in Silver King Creek, where we observed differences between rotenone-treated sites and the untreated site in overall abundance and numbers of Coleopterans, but overall there were few differences in most measures after several low dosage ($<$25-μg/L) rotenone treatments. These results contrast Darby et al. (2004) who found Coleoptera to be one of the more resilient insect groups to rotenone, and toxicity data suggest that they are one of the least-sensitive orders to rotenone (Table 1). It is possible that differences in Coleoptera abundance may have been more likely caused by differences in microhabitat than rotenone toxicity.

The direct impacts of toxicants on organisms are governed by the concentration and duration of exposure (as Paracelsus [circa 1493–1541] noted, "the right dose differentiates a poison from a remedy"). High concentrations of rotenone (125–250 μg/L rotenone) and long treatment durations (up to 48 h) have resulted in severe impacts to invertebrate assemblages (Binns 1967; Mangum and Madrigal 1999; Darby et al. 2004). Conversely, lower concentrations of rotenone (25–75 μg/L rotenone) and shorter durations ($<$18 h) have resulted in less-severe impacts (Cook and Moore 1969; Maslin et al. 1988; Trumbo et al. 2000a, 2000b; Whelan 2002).

Our results and those of previous workers (Flint et al. 1998; Whelan 2002) suggest that rotenone treatment rates between 25 and 50 μg/L for 4–8 h should eliminate trout in most circumstances and reduce impacts to aquatic invertebrate assemblages. Treating at higher doses and durations may produce a false sense of security, but it will not further increase the likelihood of eliminating trout; fish can only be killed once. Higher dosage rates will also incrementally increase the impacts to invertebrate assemblages and does not address the likely cause of treatment failure from missing occupied fish habitat (i.e., seeps, springs, headwater lakes) or improper application. However, higher doses, longer durations, or both may be required to kill fish in problematic habitat of streams (i.e., deep pools, slack water, and extensive aquatic vegetation), and these areas should be identified with strategic placement of caged sentinel fish. Our results suggest it would likely take twice as much Nusyn-Noxfish to yield the effect of CFT Legumine on trout, but Nusyn-Noxfish rotenone is likely twice as toxic to aquatic insects because of the synergist. The synergist piperonyl butoxide is widely used in commercial insecticide formulations, demonstrating its effectiveness on insects, but our data would suggest that it is much less effective as a synergist on fish. Therefore, there is little reason to use synergized formulations in trout removal projects given that it will take twice the amount of rotenone product and produce a greater impact to invertebrate assemblages.

### *Recommendations*

Based on the results presented here and in previous reviews (e.g., Finlayson et al. 2000), fisheries managers applying rotenone for fish eradication projects in mountain streams should consider the following measures to maximize treatment efficiency on fish and to minimize impacts to nontarget aquatic invertebrates: (1) apply rotenone at treatment rates between 25 and 50 μg/L, (2) operate rotenone drip stations for 4–8 h per treatment, (3) use unsynergized formulations because the synergized formulation is less toxic to fish and more toxic to aquatic insects, (4) for chemical treatments of larger drainages stage treatments with intermediate barriers and allow time between treatments for dispersal and recolonization of invertebrates to avoid potential for cumulative impacts, (5) leave headwater reaches of drainages that are above barriers and have never inhabited fish as untreated refuges for

invertebrates and a source for recolonization of downstream treated reaches, (6) neutralize rotenone downstream of the project area, (7) consider aquatic invertebrate "rescues" to probably be impractical except where treating whole or isolated basins or the presence of endangered invertebrate species, and (8) strategically use caged sentinel fish and collect water samples for rotenone content throughout the treatment area to monitor efficacy.

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