

FATE AND BEHAVIOR OF ROTENONE IN DIAMOND LAKE, OREGON, USA FOLLOWING
INVASIVE TUI CHUB ERADICATION

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Abstract: In September 2006, Diamond Lake (OR, USA) was treated by the Oregon Department of Fish and Wildlife with a mixture of powdered and liquid rotenone in the successful eradication of invasive tui chub *Gila bicolor*. During treatment, the lake was in the middle of a phytoplankton (including cyanobacteria *Anabaena* sp.) bloom, resulting in an elevated pH of 9.7. Dissipation of rotenone and its major metabolite rotenolone from water, sediment, and macrophytes was monitored. Rotenone dissipated quickly from Diamond Lake water; approximately 75% was gone within 2 d, and the average half-life ($t_{1/2}$) value, estimated by using first-order kinetics, was 4.5 d. Rotenolone persisted longer (>46 d) with a short-term $t_{1/2}$ value of 16.2 d. Neither compound was found in groundwater, sediments, or macrophytes. The dissipation of rotenone and rotenolone appeared to occur in 2 stages, which was possibly the result of a release of both compounds from decaying phytoplankton following their initial dissipation. Fisheries managers applying rotenone for fish eradication in lentic environments should consider the following to maximize efficacy and regulatory compliance: 1) treat at a minimum of twice the minimum dose demonstrated for complete mortality of the target species and possibly higher depending on the site's water pH and algae abundance, and 2) implement a program that closely monitors rotenone concentrations in the posttreatment management of a treated water body. *Environ Toxicol Chem* 2014;33:1650–1655. © 2014 SETAC

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INTRODUCTION

A rainbow trout (*Oncorhynchus mykiss*) fishery was established in formerly fishless Diamond Lake (OR, USA) in the early 1900s by the Oregon Game Commission [1,2] and became very popular beginning in the 1920s. This hatchery fish-maintained recreational fishery thrived until the 1940s, when it was discovered that the nonindigenous tui chub *Gila bicolor* had been introduced into Diamond Lake [1]. The chub population expanded and decimated the trout fishery. The chub were eradicated from the lake using powdered rotenone in 1954, trout were reintroduced into the lake, and the fishery was restored [2]. Rotenone, a phosphorylation inhibitor, is a botanical material found in roots, seeds, and leaves of various plants that are members of the bean family Leguminosae from Australia, Oceania, southern Asia, and South America [3,4]. It has been used for centuries to capture fish for food in areas where rotenone-containing plants were naturally found and is currently used in several countries as a piscicide in fish management [5,6].

The tui chub eradication lasted until 1992, when they were again found in the lake, likely from their unsanctioned use as live bait. As before, the trout fishery declined, and a second rotenone treatment was proposed [7]. The latest introduction of chub has caused major increases in cyanobacteria, changes in diatom community composition, a reduction in water transparency, increases in the proportion of rotifers, a major reduction in benthic standing crop, and virtual elimination of amphipods, gastropods, and other large-bodied invertebrates [8,9]. The lake

began experiencing severe blooms of cyanobacteria in 2001 (*Anabaena* sp. density reached 575 000 cells/mL) that exceeded those recommended for recreational contact [10]. The cyanobacteria blooms, which occasionally closed the lake to recreational contact, were likely in response to the stimulatory effects of tui chub waste products rather than concurrent reduction of phytoplankton grazing pressure associated with the loss of the large cladocerans [8]. The fish biomass in the lake at the time of the rotenone treatment in 2006 was composed almost exclusively of tui chub (99.9% tui chub), and estimates of chub population in the lake varied from 7.6 million to 23 million fish [9].

The loss of the trout fishery and the public health threat from the cyanobacteria blooms prompted the Oregon Department of Fish and Wildlife to treat Diamond Lake with rotenone in September 2006 to eradicate the tui chub [7]. However, concerns were expressed about the safety of rotenone, particularly its persistence in the aquatic environment because of the extensive recreational use of the lake, the numerous shallow drinking water wells around the lake, and the discharge of treated water into the outlet creek that eventually drains into the North Umpqua River (OR, USA). To protect public health, the US Environmental Protection Agency (USEPA) [3] determined that there should be no contact with treated water until rotenone residues subside to <90 ppb and no use of treated water for drinking until rotenone residues subside to <40 ppb. Rotenone has low to moderate mobility in soil and sediment, has a relatively low potential for bioconcentrating in aquatic organisms, and is unstable in the environment, with hydrolysis and photolysis half-lives measured in days and hours, respectively [11]. Although rotenone degrades quickly in temperate aquatic environments [12–21] compared with rotenolone, new restrictions on its use as a piscicide—including

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re-entry intervals, monitoring requirements, and chemical deactivation of treated flowing waters—are dependent on rotenone concentrations [3,11]. Thus, comprehensive posttreatment monitoring of rotenone concentrations in Diamond Lake occurred following the 2006 treatment [7].

The objectives of the present study were to characterize the dissipation of rotenone from Diamond Lake water, sediment, and macrophytes and to relate these to the successful eradication of tui chub and the posttreatment management of Diamond Lake.

MATERIALS AND METHODS

Study site

Diamond Lake, located near the crest of the Oregon Cascade Range in the Umpqua River Basin (Figure 1), was treated with rotenone in 2006 to eradicate the nonindigenous tui chub and improve water quality [8,9]. The subalpine lake typically stratifies in July and August and is ice-covered from December to April. The relatively shallow (6.9 m mean depth), 1226-ha lake sits at an elevation of 1580 m and has a 14.8 m maximum depth and a volume of $84.0 \times 10^6 \text{ m}^3$ [8]. The 136-km² watershed area has 2 main inlet streams, Short Creek and Silent Creek, that provide 58% of the inflow to the lake. The balance of water input comes from precipitation (32%) and groundwater (10%) [9]. A single stream, Lake Creek, leaves the lake through a head-gate on the north end of Diamond Lake (Figure 1) and then enters the upper North Umpqua River after flowing through Lemolo Reservoir.

Rotenone application

Diamond Lake was treated using a mixture of the liquid Prentox Prenfish Toxicant (USEPA reg. no. 655-422) and

Prentox Rotenone Fish Toxicant Powder (USEPA reg. no. 655-691). The liquid is formulated to contain 5% w/w active rotenone, and the powder varies naturally from 7.5% to 9.4% (mean of 8.6%) w/w active rotenone. To determine the dosage required for eradication, tui chub sensitivity to rotenone was tested in on-site bioassays in 2005 and 2006 using water from Diamond Lake. Concentrations of Prenfish Toxicant tested ranged from 0.5 mg/L (0.025 mg/L active rotenone) to 2.0 mg/L (0.1 mg/L active rotenone). The tests indicated that at ambient water temperature (12–15 °C) and pH (7.1–7.2), Prenfish Toxicant concentrations $\geq 0.5 \text{ mg/L}$ were lethal to chub within 2 h, and 2.0 mg/L was lethal within 1.5 h. The lowest concentration that produced 100% chub mortality in Diamond Lake water, and thus the minimum effective dose, was 0.025 mg/L (25 $\mu\text{g/L}$) active rotenone. Rotenone is an unstable compound in temperate waters, degrading through hydrolysis [12], photolysis [17,18], metabolism [19–21], and adsorption onto particulate matter [13], resulting in aqueous $t_{1/2}$ values of 0.6 d to 7.7 d [15]. To account for this likely loss of rotenone and to ensure that the minimum effective dose was present in Diamond Lake during the 2-d to 3-d lake application/mixing period, the treatment dose was increased 4-fold from the minimum effective dose to 100 $\mu\text{g/L}$ active rotenone; Finlayson et al. [11] recommend treating at a minimum of twice the minimum effective dose.

The Diamond Lake water level was lowered 2.65 m from November 2005 to July 2006, reducing the lake volume and the amount of rotenone required by approximately 37% and ensuring that no discharge from the lake would occur immediately after the rotenone application. Diamond Lake became destratified by 1 September following the fall turnover. On 13 September and 14 September 2006, the $53 \times 10^6 \text{ m}^3$ volume of Diamond Lake was treated with $5.837 \times 10^3 \text{ kg}$ active rotenone, resulting in an estimated initial maximum treatment dose (based on dilution) of 110 $\mu\text{g/L}$ from 35 204 L of Prenfish Toxicant applied to the shallower (<6.1 m) areas and 48 590 kg of Rotenone Fish Toxicant Powder applied to the deeper (>6.1 m) areas. Nine large-capacity (2177 kg payload) pontoon boats applied the majority of the rotenone to the lake over approximately 10 h during the 2-d period using the gasoline-powered, pump-driven semiclosed application systems described by Finlayson et al. [11]. Although chub did not inhabit the 2 inlet streams, the terminal flowing portions (<1.5 km) were treated with drip cans discharging Prenfish Toxicant to prevent the occurrence of untreated water in the lake, which could provide a refuge for fish during treatment.

Sentinel tui chub were placed in cages at depths ranging from 0.3 m to 10.7 m at 12 sites throughout the lake before treatment. Tui chub began dying at the surface in Diamond Lake within several hours after beginning the rotenone application on 13 September 2006, and all (100% mortality) sentinel fish were dead when retrieved on 15 and 16 September. The head-gate to Lake Creek was opened on 30 November 2006 after repeated chemical analyses and toxicity testing with caged trout confirmed that active rotenone was not present in the lake. Rainbow trout were stocked in Diamond Lake starting in June 2007, and this stocking has continued annually. The lake achieved full pool on 12 July 2007.

Surface water, well, sediment, vegetation, and algae sampling

Surface water samples for rotenone and rotenolone analysis were collected in clean 1-L amber glass bottles with Teflon[®]-lined caps from various depths at 12 sites that were identified by Northing and Easting measurements (Figure 1). Sample sites were repeatedly sampled using a boat and a GPS unit. Shallow

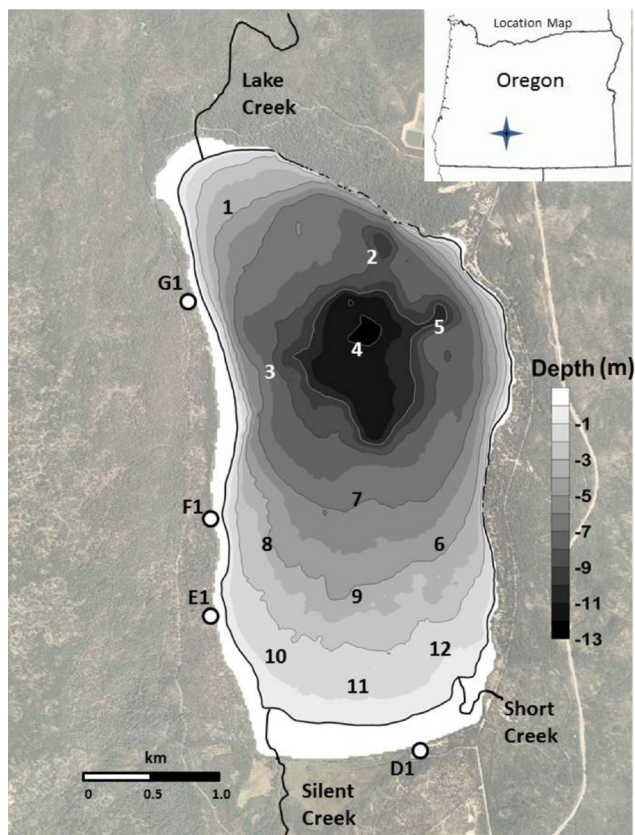


Figure 1. Diamond Lake (Oregon, USA) map with surface water (sites 1–12) and groundwater monitoring well (sites D1–G1) sampling sites. The black line delineates the shoreline at maximum drawdown.

(<8 m) sites (sites 1, 6, and 8–12) were sampled only at mid-depth and medium depth (>8, <12 m). Sites 2, 3, 5, and 7 were sampled 1 m below the surface and 1 m above the bottom, and the sole deep (>12 m) site (site 4) was sampled at the surface, mid-depth, and bottom. Once anchored at the site, a weighted Tygon[®] (chemically resistant and plasticizer-free) tube marked with depth increments was lowered to the desired sample depth. Water was sampled through the tubing using a RolaTec peristaltic model MP-V400 pump, directly into the sample bottle. The water collection apparatus was rinsed twice with site water before the site water was collected to avoid contamination. Care was taken to exclude air space in the bottles and caps. Water samples were collected before treatment in August 2006 and at 2 d, 11 d, 18 d, 25 d, 32 d, 39 d, and 46 d posttreatment.

Four groundwater observation wells (D1–G1; Figure 1), with hydrological characteristics described in detail by Eilers [22], were sampled for rotenone analysis in a manner similar to Diamond Lake surface water by lowering Tygon tubing attached to a peristaltic pump into the well. Water was pumped from the well into a receiving bucket until the water temperature and conductivity had stabilized, which usually involved removing approximately 3 times the volume of the well prior to sampling. Although the pumping method was slightly greater than specified by Puls and Barcelona [23], the rates were still low enough to be classified as a minimal drawdown method of sampling. Samples were collected before treatment in August 2006 and at 25 d, 32 d, and 39 d posttreatment.

Sediment (48 d posttreatment) and aquatic macrophyte (46 d posttreatment) samples were collected for rotenone analysis using a stainless steel petite PONAR sampler (Wildco model 1728-G42) from 4 random sites near the shoreline. Macrophyte samples were separated by hand from sediment and processed separately. All samples were placed into clean Ziploc[®] bags.

The abundance of phytoplankton in Diamond Lake was monitored monthly from site 4, 1 m below the water surface, using a RolaTec peristaltic model MP-V400 pump. Taxonomic analyses were conducted by Aquatic Analysts (Friday Harbor, WA, USA). Phytoplankton samples were preserved in Lugol's solution, subsamples were permanently mounted onto slides, and measured transects were scanned at 1000× magnification using a phase-contrast compound microscope. Counting was generally limited to 100 cells per sample. This is a modification of American Public Health Association method 10200F.2.c (high-magnification methods) [24]. Although the method is somewhat nonstandard, it is consistent with the methodology used on Diamond Lake studies since 1992. Biovolume estimates were calculated for each algal unit (for filamentous algae, the biovolume unit was standardized to 100-μm length of filament) based on measurements of average algal length and diameter.

To ensure sample identity, all samples were identified by a unique number, placed in a cooler with ice immediately after collection, and shipped overnight to the laboratory for analysis. Samples were accompanied by chain-of-custody forms documenting the complete sequence of transfer from collection to analysis.

Rotenone extraction and analysis

Residues of rotenone and rotenolone were analyzed by liquid chromatography–mass spectrometry (LC/MS) in sediment and vegetation samples and by liquid chromatography–tandem mass spectrometry (LC/MS-MS) in water samples by the California Department of Fish and Game Water Pollution Control Laboratory, Rancho Cordova [25], using the methods, instru-

ments, and settings described by Vasquez et al. [16]. In summary, rotenone standard (CAS no. 83-79-4) was purchased from ChemService, and rotenolone (12ab-hydroxyrotenone) came from a degraded rotenone standard because no commercial sources are available. A synthesized rotenolone standard (University of California, Berkeley, CA, USA) was used to validate the rotenolone fraction in the degraded standard [16,25]. If matrix interferences prohibited direct injection of aqueous samples, preconditioned C₁₈ solid-phase extraction was used as a cleanup procedure to remove interference using the procedure described by Vasquez et al. [16,25]. Sediment and vegetation samples (10 g dry wt) were extracted employing accelerated solvent extraction with acetonitrile:methylene chloride (50:50 [v/v]) using the methods described in Vasquez et al. [16,25]. Samples were processed within 1 d to 7 d of arrival. The minimum detection level was 0.05 μg/kg or 0.05 μg/L, and the reporting limit was 0.1 μg/kg or 0.1 μg/L for both rotenone and rotenolone. Laboratory accuracy (recovery) was determined using samples enriched with target compounds (matrix spikes); recovery of rotenone and rotenolone from water varied from 85.7% to 114% and from 75.5% to 127%, and recovery of rotenone and rotenolone from sediment varied from 70.9% to 96.3% and from 71.0% to 85.9%, respectively.

Dissipation of rotenone and rotenolone

Dissipation rates for rotenone and rotenolone in Diamond Lake water were estimated assuming first-order kinetics [26] in which the short-term rate constant k (d⁻¹) between 2 sampling events was calculated from the equation

$$k = (\log_e c_0 - \log_e c) / t \quad (1)$$

where c_0 is the initial concentration and c is the concentration (μg/L) at time t (d). A plot of the logarithm of the concentration of the chemical versus time is a straight line

$$\log_e c = -kt + \log_e c_0 \quad (2)$$

The average rate constant k (d⁻¹) was also obtained from the slope of the line, estimated by linear regression analysis. The $t_{1/2}$ values were estimated from k using the equation

$$t_{1/2} = \log_e 2 / k = 0.693 / k \quad (3)$$

Because the dissipation of rotenolone is confounded by the ongoing transformation of rotenone to rotenolone until the rotenone is exhausted, $t_{1/2}$ values of rotenolone were calculated only after complete rotenone dissipation.

RESULTS

All pretreatment water samples from the lake and groundwater observation wells had undetectable levels (<0.1 μg/L) of rotenone and rotenolone when sampled on 29 August 2006. During the 2-d application, lake water temperature varied from 17 °C to 18 °C, and the pH was elevated to 9.7 because of a phytoplankton bloom (Figure 2). Water temperature slowly declined to 10 °C by mid-October. Following the application, rotenone dissipated quickly from Diamond Lake surface water (Supplemental Data, Table S1), and most of the rotenone was gone within 18 d posttreatment; small amounts persisted until 32 d posttreatment (Figure 3). At 2 d posttreatment, the mean ± standard deviation (SD) concentration of rotenone in Diamond Lake surface water was 28.4 ± 6.6 μg/L (Supplemental Data,

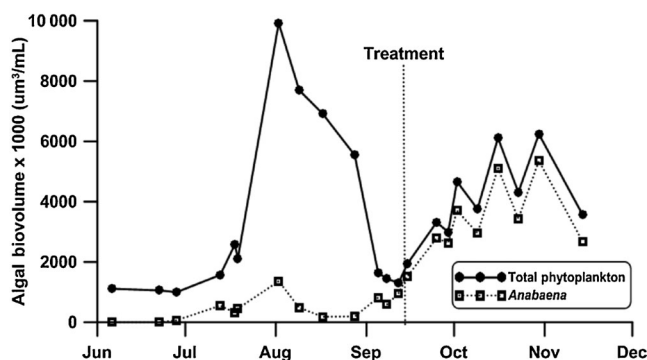


Figure 2. Biovolume of total phytoplankton and *Anabaena* sp. in Diamond Lake (2006).

Table S1). Approximately 25% of the calculated target dose (based on known mass in known volume) of 110 µg/L remained, yielding a short-term rotenone $t_{1/2}$ value of 1.02 d (Equations 1 and 3). At 11 d posttreatment, the mean \pm SD rotenone concentration had dissipated to 7.4 ± 3.1 µg/L. Rotenone dissipation appeared to occur in 2 stages, decreasing from application to 18 d posttreatment, increasing from 18 d to 25 d posttreatment, and then decreasing from 25 d posttreatment at all monitored sites (Figure 3). The average rotenone $t_{1/2}$ value was 4.5 d ($r = 0.93$; Equations 2 and 3) for 32 d after treatment.

The mean \pm SD concentration of rotenolone (21.2 ± 7.3 µg/L) in the Diamond Lake surface water was similar to rotenone at 2 d posttreatment; unlike rotenone, however, the mean \pm SD rotenolone (21.3 ± 6.4 µg/L) concentration at 11 d posttreatment had not changed (Supplemental Data, Table S1). The dissipation of rotenolone also appeared to occur in 2 stages, decreasing to 18 d posttreatment, increasing from 18 d to 25 d posttreatment, and then decreasing from 25 d posttreatment at all monitored sites (Figure 4). Rotenone concentrations were present until 39 d posttreatment, but rotenolone concentrations in Diamond Lake's surface water persisted for 46 d after treatment and likely beyond. The short-term rotenolone $t_{1/2}$ value was 16.2 d (Equations 1 and 3) between 39 d and 46 d posttreatment.

The 4 groundwater observation wells (Figure 1) adjacent to Diamond Lake remained free of rotenone (<0.1 µg/L) and rotenolone (<0.1 µg/L) when sampled 25 d, 32 d, and 39 d after treatment. The aquatic vegetation and bottom sediment had no

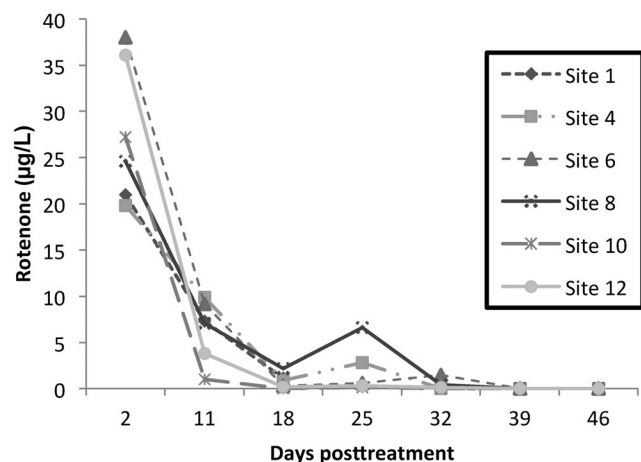


Figure 3. Rotenone dissipation profiles in Diamond Lake (2006).

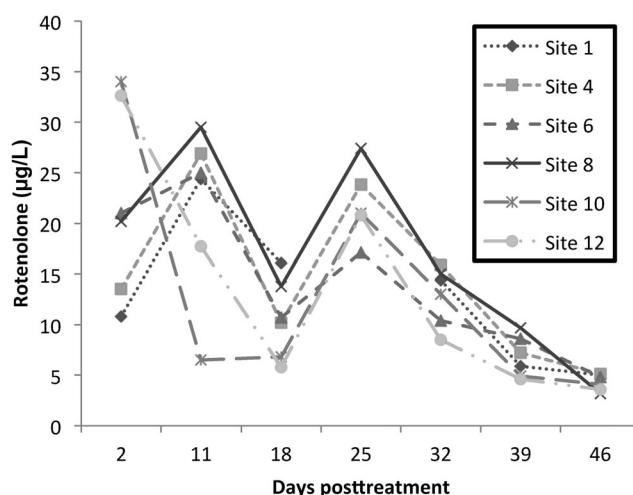


Figure 4. Rotenolone dissipation profiles in Diamond Lake (2006).

detectable residues of rotenone (<0.1 µg/kg) or rotenolone (<0.1 µg/kg) when sampled at 46 d and 48 d after treatment.

DISCUSSION

Rotenone degraded quickly from Diamond Lake as a result of the relatively warm water temperature of 17 °C to 18 °C and an elevated pH of 9.7 from the phytoplankton bloom (Figure 2 [9]). At 2 d posttreatment, only approximately 45% of the rotenone applied could be accounted for by adding together the average rotenone (28.4 µg/L) and metabolite rotenolone (21.2 µg/L) concentrations in the water ($49.6/110$ µg/L), and the rotenolone concentration was nearly as high as that of rotenone. This is consistent with the findings of Thomas [12], who found that 50.1% of the rotenone added to warm (25 °C) water at pH 9 transformed into rotenolone within 30 d. The addition of the 2 compounds shortly following application should be representative of the rotenone mass applied. Rotenone is an unstable compound in water and dissipates through hydrolysis, photolysis, and metabolism to the intermediate metabolite rotenolone [17–21]. Hydrolysis $t_{1/2}$ values vary from 2.0 d at a pH of 9 to 12.6 d at a pH of 5 [12]; photolysis $t_{1/2}$ values vary from 1.4 h [17] to 8.2 h [18]; and bacteria [20], fish [19], and mammals [21] metabolize rotenone to rotenolone.

Approximately 75% of the rotenone applied had dissipated by 2 d posttreatment (50% by 1 d + 25% by 2 d), yielding a short-term rotenone $t_{1/2}$ value of 1.02 d. Rotenone $t_{1/2}$ values are inversely related to water temperature and pH values; for waters of similar pH (8.8–9.5) and temperature (18–27 °C), $t_{1/2}$ values of 0.65 d to 1.7 d have been documented in California waters [15]. Campbell and Rueppel [27] reported a rotenone $t_{1/2}$ value of 1.2 d in Lake Haussmann with a pH of 9.8 and a temperature of 19 °C, and Gilderhaus et al. [14] reported a rotenone $t_{1/2}$ value of approximately 0.58 d (pH of 8.9 and temperature of 24 °C) in an experimental pond in Wisconsin.

Diamond Lake water had an average $t_{1/2}$ value of 4.5 d for rotenone (32 d posttreatment). Recently, Vasquez et al. [16] determined an average rotenone $t_{1/2}$ value of 5.6 d in water from Lake Davis, California. The average $t_{1/2}$ values for rotenone were similar for Lake Davis and Diamond Lake likely because of similar water temperatures at application in early September (17–20 °C) and temperature declines (9–10 °C) within 30 d posttreatment. During this period, because temperature declined approximately 10 °C in both water bodies, the dissipation of both

rotenone and rotenolone would be expected to decline 2- to 3-fold, consistent with the temperature coefficient (Q_{10}). Rotenone was gone from Diamond Lake by 39 d and from Lake Davis by 34 d. Finlayson et al. [15] noted that rotenone $t_{1/2}$ values were typically inversely related to temperature for a variety of California waters.

Although the dissipation rates of rotenone from the 2 lakes were similar, rotenolone in Lake Davis was nearly gone (detectable at 1 of 10 sites) by 41 d [16] but was still present at all sites at 46 d, and likely beyond, in Diamond Lake. Finlayson et al. [15] noted that rotenolone concentrations appeared to parallel rotenone concentrations in water and were typically not found in the absence of rotenone. We were unable to determine the true dissipation of rotenolone in either body until rotenone itself had dissipated because of the ongoing transformation of rotenone to rotenolone. However, this biased the results toward longer half-life values than were likely present during rotenone dissipation because there were corresponding decreases in water temperature. We calculated a short-term $t_{1/2}$ value (Equations 1 and 3) for Lake Davis (between 34 d and 41 d posttreatment) after rotenone dissipation using the supplemental data of Vasquez et al. [16]. Despite similar water temperatures, rotenolone dissipation in Diamond Lake (between 39 d and 46 d posttreatment) lagged behind that in Lake Davis by 5-fold compared with the short-term $t_{1/2}$ values for rotenolone (16.2 d and 3.2 d, respectively).

Rotenone and rotenolone concentrations were not found in the Diamond Lake groundwater monitoring wells. Rotenone is not considered a likely groundwater contaminant because of its unstable nature in the environment and high sediment sorption coefficient ($K_d > 100$) for soils with high silt or organic content [13]. Rotenone has not been detected in over 26 wells monitored in California [15]. Rotenone and rotenolone were also absent from vegetation and sediment at 46 d to 48 d posttreatment even though rotenolone was still present in the water column. The lack of these residues in the sediment was expected given their quick dissipation from Diamond Lake water, and previous monitoring studies have typically not found these persisting longer than 60 d posttreatment [15]. However, Vasquez et al. [16] did find rotenone in the sediment of Lake Davis for 6 mo. The differences in sediment residues from the 2 lakes may be because of the *Anabaena* sp. and other phytoplankton interfering with rotenone and rotenolone dissipation from Diamond Lake. There are no previous data for rotenone and rotenolone residues in macrophytes.

There was $>25 \mu\text{g/L}$ rotenone (minimum lethal dose for 2-h exposure) present in Diamond Lake at 2 d posttreatment. There is strong evidence, based on the rotenone monitoring data and the complete mortality of the sentinel tui chub, that this species was eradicated from Diamond Lake in September 2006. Trap netting, beach seining, and backpack and boat electrofishing efforts since treatment (2006–2012) have not found tui chub (Oregon Department of Fish and Wildlife, unpublished data). Had only the minimum effective dose ($25 \mu\text{g/L}$) been applied and not 4-fold that dose, it is likely that rotenone would have degraded 75% to approximately $6.25 \mu\text{g/L}$ prior to complete mixing in Diamond Lake, possibly resulting in an incomplete kill of tui chub. This corroborates the recommendation of Finlayson et al. [11] that a concentration of at least twice the minimum effective dose be used to compensate for variables (i. e., sunlight, high pH, warm temperature, metabolism, and turbidity) that will degrade rotenone while it is mixing in lakes.

We anticipated that both rotenone and rotenolone would dissipate quickly from Diamond Lake given the warm

temperature and high pH water quality conditions. However, the dissipations of both chemicals (Figures 3 and 4) occurring in 2 stages and the lagging rotenolone dissipation were not expected. A 4-fold increase in rotenone (Figure 3) and a 3-fold increase in rotenolone (Figure 4) concentrations between 18 d and 25 d posttreatment (Supplemental Data Table S1) suggest a release of these compounds back into water from a source, possibly decaying *Anabaena* sp., phytoplankton, and/or fish biomass during this period. Rotenone's high octanol–water partition coefficient ($\log K_{OW} = 4.10$ [28]) suggests that it would strongly bioconcentrate in fish; similar data are not available for rotenolone. However, rotenone is metabolized (likely to rotenolone and derivatives) and eliminated by fish, producing a low bioconcentration factor (tissue residue:water concentration) of 27.6 in whole fish tissue [19]. In previous studies reviewed by Jarvinen and Ankley [29], low rotenone residues (0.22–1.08 mg/kg) were found in fish killed by rotenone. The tui chub biomass in Diamond Lake was estimated at 357 000 kg [9] and potentially may have bound 0.385 kg rotenone ($357\ 000 \text{ kg} \times 1.08 \text{ mg rotenone/kg fish}$). However, the 0.385 kg of rotenone diluted in the $53 \times 10^6 \text{ m}^3$ volume of Diamond Lake would result in an increase of only $0.007 \mu\text{g/L}$, several orders of magnitude lower than the $1.4 \mu\text{g/L}$ increase in mean rotenone concentration found between 18 d and 25 d posttreatment. Similar information on the potential of algae to bioaccumulate rotenone is lacking, but is likely significant given rotenone's high K_{OW} , the oily content of algae, and the phytoplankton bloom during and following the rotenone application (Figure 2). Mahakant et al. [30] found that 2 species of *Anabaena*, the dominant genus associated with the posttreatment bloom in Diamond Lake (Figure 2), contained 15.7% to 22.4% oil (dry wt), and Kuritz [31] found the oil content of algae to be up to 77% (dry wt). Anecdotally, there have been 2 instances of fish killed in streams after rotenone should have been flushed from the system; it was suspected that aquatic vegetation (i.e., mosses and palms) acted as sponges that released rotenone back into the water after the treatment ended (B. Finlayson, personal communication). One explanation for the behavior of rotenone and rotenolone in Diamond Lake is that the *Anabaena* sp. and other phytoplankton absorbed rotenone and rotenolone and released them back into the water over time.

The release of rotenone back into water may affect the posttreatment management of a treated water body. Although the mean increase in rotenone between 18 d and 25 d posttreatment was only $1.4 \mu\text{g/L}$, 1 site in Diamond Lake had an increase of $4.4 \mu\text{g/L}$, and concentrations may have been higher at other sites or sampling intervals. The new use restrictions prohibit recreational contact until rotenone concentrations are $<90 \mu\text{g/L}$ and require deactivation of discharged treated waters until rotenone concentrations are $<2 \mu\text{g/L}$ [3,11]. Neither activity was an issue for Diamond Lake because the highest concentration detected in water was less than half the minimum allowed for recreational contact, and water was not discharged into Lake Creek until 30 November, more than 1 mo after rotenone residues had disappeared. A well-designed rotenone monitoring program, especially if treatment is at doses higher than these water use restrictions, will help ensure regulatory compliance.

RECOMMENDATIONS

The present study demonstrates that rotenone can degrade very quickly under warm, alkaline pH conditions and that phytoplankton may confound rotenone and rotenolone decay by acting as a sponge, releasing both materials back into the water

over time. To overcome the loss of rotenone under these conditions, treat at a minimum of twice the minimum effective dose for the target species or possibly higher, depending on the site's water quality conditions (i.e., pH, temperature, turbidity, algae abundance). Regulatory compliance now uses rotenone concentrations as criteria for requiring deactivation of discharged treated water (>2 ppb rotenone) and the resumption of public contact (<90 ppb rotenone) and water consumption (<40 ppb rotenone) [3,11]. To ensure regulatory compliance, implement a program that closely monitors rotenone concentrations in the posttreatment management of a treated water body.

SUPPLEMENTAL DATA

Table S1 (16 KB DOC).

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