Piscicides and Invertebrates: After 70 Years, Does Anyone Really Know?

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ABSTRACT: The piscicides rotenone and antimycin have been used for more than 70 years to manage fish populations by eliminating undesirable fish species. The effects of piscicides on aquatic invertebrate assemblages are considered negligible by some and significant by others. This difference of opinion has created contentious situations and delayed native fish restoration projects. We review the scientific evidence and report that short-term (< 3 months) impacts of piscicides to invertebrate assemblages varied from minor to substantial and long-term (> 1 year) impacts are largely unknown. Recovery of invertebrate assemblages following treatments ranged from a few months for abundances of common taxa to several years for rarer taxa. Variation in reported effects was primarily due to natural variation among species and habitats and a lack of adequate pre- and post-treatment sampling which prevents determining the true impacts to invertebrate assemblages. The factors most likely to influence impacts and recovery of aquatic invertebrate assemblages following piscicide treatments are: (1) concentration, duration, and breadth of the piscicide treatment; (2) invertebrate morphology and life history characteristics, including surface area to volume ratios, type of respiration organs, generation time, and propensity to disperse; (3) refugia presence; and (4) distance from colonization sources.

Piscicidas e invertebrados: después de 70 años ¿Realmente alguien sabe?

RESUMEN: Los piscicidas rotenona y antimicina han sido utilizados por más de 70 años para manejar poblaciones de peces, eliminando especies indeseables. Para algunos autores los efectos de los piscicidas en las asociaciones de invertebrados acuáticos son considerados como insignificantes sin embargo, para otros, son importantes. La diferencia entre las opiniones ha creado una situación tirante, retrasando así los proyectos de restauración de peces nativos. Revisando la evidencia científica, se encontró que en el corto plazo (<3 meses) los impactos de los piscicidas en las asociaciones de invertebrados varió de menor a sustancial, y en el largo plazo (>1 año) los impactos son básicamente desconocidos. Tras recibir los tratamientos, la recuperación de dichas asociaciones fue de pocos meses para los taxa más abundante hasta varios años para los taxa más raros. La variación en los efectos reportados se debió principalmente a la variación natural entre especies y hábitats y a la falta de un adecuado muestreo pre y post-tratamiento. Los factores que más probablemente determinen el impacto y recuperación de las asociaciones de invertebrados después del tratamiento con piscicidas son: (1) concentración, duración y espectro del tratamiento de piscicida; (2) la morfología de los invertebrados así como las características de su historia de vida, incluyendo la razón superficie-volumen, tipo de órganos respiratorios, tiempo generacional y propensión a la dispersión; (3) presencia de refugios; y (4) distancia hacia las áreas de colonización.

INTRODUCTION

The piscicides rotenone and antimycin A (hereafter antimycin) have been used for more than 70 years to manage fish populations by eliminating undesirable fish species (McClay 2000). While piscicides are intended to control and eradicate fish, they can also be toxic to non-target aquatic biota, such as invertebrates and amphibians. Impacts on aquatic invertebrates are a concern because of their role in ecosystem processes and their importance as food sources for fish. A popular belief among fisheries professionals has generally been that impacts to invertebrates are minimal and short-term. This view is frequently repeated in both professional society publications (e.g., Finlayson et al. 2005), sportsmen-oriented publications (e.g., Williams 2002, 2007), and in piscicide project planning documents. Alternatively, others, such as the Center for Biological Diversity (2003), have claimed that piscicides cause irrevocable damage. This difference of opinion has led to litigation and caused delays in native fish restoration projects (Finlayson et al. 2005). We suggest that the true impacts of rotenone and antimycin on invertebrate populations are not well known. The objective of this article is to review published studies on the effects of rotenone and antimycin on invertebrate assemblages. Lastly, we provide some recommendations on sampling schemes to allow for more robust analyses of piscicide effects.

HOW PISCICIDES WORK

Antimycin and rotenone belong to a class of chemicals known as oxidative phosphorylation inhibitors or uncouplers. These affect toxicity through disrupting cellular respiration (energy generation) in the mitochondria, but at slightly different sites in the respiratory chain. Rotenone is a naturally occurring compound found in many plants within the family Leguminosae. Rotenone concentrations of 25 parts per billion (ppb or µg/L) or higher can be toxic to most fish and some invertebrates (Ling 2003). Rotenone may be detected by fish and fish avoidance may occur. Antimycin is an antibiotic produced by several species of *Streptomyces* bacteria (Harada and Tanaka 1956). Most fishes can be killed by antimycin concentrations of 20 parts per billion or less and fish are unable to detect antimycin. Antimycin has been reported to be effective in small streams, shallow ponds and alpine lakes, whereas rotenone is reported to be effective in most situations including large rivers and deep lakes (Finlayson et al. 2000).

There are three commonly available commercial forms of rotenone: two liquids containing either 5% active ingredient or 2.5% active ingredient with a 2.5% synergist, and a powder containing 5% rotenone. These products are generally applied at a treatment rate of 1–5 mg/L (ppm) which yields active rotenone concentration of 0.025–0.25 mg/L (25–250 ppb or µg/L). In the literature, values are generally reported as treatment rate concentrations of 2.5 or 5% rotenone products. In this review, we attempted to standardize rotenone concentrations to ppb of active rotenone, e.g., 5 mg/L of 5% rotenone solution = 250 ppb active rotenone. Currently, only one form of antimycin is commercially available, Fintrol® (11% active ingredient) and application rates are reported in ppb or equivalent µg/L active antimycin.


OREGON DEPARTMENT OF FISH AND WILDLIFE

A barge loaded with rotenone and manpower heads out into Diamond Lake, Oregon, 21 September 1954. The 1,200 surface hectare lake was treated with 90,718 kilograms, plus 1,041 liters of liquid rotenone for treatment of tributary streams and for aerial spraying of a marsh area.


KIRK PATTEN

A rotenone drip station used by New Mexico Department of Game and Fish on Costilla Creek in September 2008. CFT legumine (5% rotenone) was applied at a constant rate for four hours to obtain an initial concentration of 50 ppb active rotenone. The project was part of a Rio Grande cutthroat trout (*Oncorhynchus clarki virginalis*) restoration project.

ROTENONE EFFECTS TO INVERTEBRATES

Laboratory results were summarized from Engstom-Heg et al. (1978). Twenty-two field studies were reviewed to assess the effects of rotenone on aquatic invertebrate assemblages. Thirteen of these studies were conducted in lentic systems (Table 2) and nine studies were conducted in lotic systems (Table 3). Rotenone concentration and treatment duration varied widely among studies. Lower concentrations were < 50 ppb (10 studies) and higher concentrations were > 100 ppb (7 studies), but not all studies provided information on the concentration of rotenone used and only one study (Trumbo et al. 2000) reported that actual concentrations were verified by field or laboratory analyses.

Rotenone: Laboratory Studies

Aquatic invertebrates have a wide range of sensitivity to rotenone, with 96 h 50% lethal concentration (LC50) values ranging down to 2 ppb (Pesticide Management Education

Program 1993). A review of published laboratory toxicity tests (Table 1, also see Ling 2003) showed several general results: (1) there has been little rotenone toxicity work on lotic aquatic invertebrates; (2) there is a wide range of sensitivity within and among taxonomic groups; (3) benthic invertebrates appear less sensitive than planktonic invertebrates; (4) smaller invertebrates appear more sensitive than larger invertebrates; (5) aquatic invertebrates that use gills to extract aqueous oxygen appear more sensitive than invertebrates that acquire aqueous oxygen cutaneously through lamellae or spiracles, use respiratory pigments, or that can breathe atmospheric oxygen; and (6) mortality was typically near 100% for rotenone concentrations of 50 to 75 ppb for lotic invertebrates and > 150 ppb for many lentic taxa depending on the exposure time. Effects appear not only related to concentration and duration, but also seem largely influenced by animal surface-area-volume ratios, with small animals like zooplankton being more susceptible than thick-bodied benthic invertebrates.

Rotenone: Lentic Studies

Rotenone effects on invertebrates in lentic habitats have been studied since the 1940s (Table 2). The results of these studies have been highly variable, with much of this variation likely related to rotenone dosage (concentration x duration) differences. Considerable variation in reported effects also appears related to the intensity, or lack thereof, of pre- and post-treatment sampling. Pre-treatment invertebrate sampling varied from a single survey to more than a year of pre-treatment sampling. Post-treatment invertebrate sampling varied from a single post-treatment sample to up to four years of post-treatment sampling. Reported impacts were generally less for studies that conducted less sampling.

More lentic studies reported greater rotenone effects on zooplankton than on benthic organisms, with most of these studies concluding that zooplankton assemblages were significantly reduced in both numbers and diversity (Table 2). More studies reported on changes in abundance than changes in species composition. Studies that have evaluated effects on benthic organisms (e.g., Cushing and Olive 1957; Houf and Campbell 1977; Koksvik and Aagaard 1984; Melaas et al. 2001) reported small differences in total benthic invertebrate abundance or biomass between pre- and post-treatment samples, with effects on Chironomidae, likely the most dominant organism, being greatest.

Recovery of zooplankton following rotenone treatments was most often reported in terms of organism abundance. Recovery to pre-treatment abundances ranged from 1 month to 3 years. Rotifer and Copepoda assemblages appeared to recover quicker than Cladoceran assemeblages (Brown and Ball 1943; Anderson 1970; Beal and Anderson 1993). Kiser et al. (1963) reported that 42 species extirpated immediately following treatment returned within 5 months. The three studies that evaluated benthic invertebrate assemblage recovery reported similar assemblages to control ponds (Houf and Campbell 1977); within 6 months (Blakely et al. 2005) and no differences between pre- and post-treatment samples within 1 year of treatment (Melaas et al. 2001).

Rotenone: Lotic studies

Study of rotenone impacts on aquatic invertebrates in rivers started in the 1960s. The majority of early studies were of short duration with little or no pre-treatment sampling and a year or less of post-treatment sampling (Table 3). Among the river studies we reviewed, three studies collected no pre-treatment data, five studies collected samples immediately before treatment, and a single study collected samples a year before treatment. Post-treatment sampling was similarly variable, with few studies collecting samples for more than a year post-treatment. Exceptions to this were Mangum and Madrigal (1999), Whelan (2002), and Hamilton et al. (2009), who collected several years of post-rotenone treatment data.

Table 1. Summary of laboratory derived rotenone tolerances (ppb hour = ppb of rotenone • duration [hour]) of selected aquatic invertebrate taxa. Summarized from Engstom-Heg et al. (1978) and Finlayson et al. (2010)*.

Low Tolerance (1,000–6,000 ppb hour)	**Intermediate tolerance (6,000–16,000 ppb hour)**	**High tolerance (1,600–24,000 ppb hour)**
Diptera	Diptera	Coleoptera
Simuliidae	Chironomidae	Elmidae
	Tipulidae: *Antocha*	Ephemeroptera
Ephemeroptera	Ephemeroptera	Leptophlebiidae: *Paraleptophlebia*
Baetidae: *Baetis tricaudatus**	Ephemerellidae: *Ephemerella*	
Heptageniidae *Rhithrogena morrisoni**	Heptageniidae	
Plecoptera	Plecoptera	Plecoptera
Perlidae *Claassenia sabulosa**	Chloroperlidae	Pteronarcyidae: *Pteronarcys*
Perlodidae *Oroperla barbara**		Megaloptera
		Corydalidae
Trichoptera	Trichoptera	Trichoptera
Psychomyiidae: *Psychomyia*	Limnephilidae	Glossosomatidae: *Glossosoma*
Hydropsychidae: *Arctopsyche grandis** *Hydropsyche**	Philopotamidae	Hydropsychidae: *Hydropsyche* *Cheumatopsyche*
Rhyacophilidae: *Rhyacophila*		Odontoceridae

Table 2. Field studies on the effects of rotenone on lentic invertebrates.

Location	Study year	Rotenone treatment	Pre-treatment sampling	Post-treatment sampling	Observed change in aquatic invertebrate assemblages	Citation
Third Sister Lake, MI	1943	5 mg/L unknown solution		Bimonthly	Zooplankton, leeches, and Odonata greatly reduced	Brown and Ball 1943
Reservoir 4 and Smith Lake, CO	1954	1 mg/L 5% rotenone solution = 50 ppb	4 Ekman dredge samples, 2 weeks prior	Biweekly Ekman dredge samples for 1 yr	Few negative effects to Chironomidae	Cushing and Olive 1957
Salbo and Holm lakes, Sweden	1958 1956	0.5–0.6 mg/L 5% rotenone solution = 25–30 ppb	Immediately prior	Immediately after	Most zooplankton and benthic fauna were killed	Almquist 1959
Fern Lake, WA	1960	0.5 mg/L 5% rotenone solution = 25 ppb	Biweekly for 2 yrs prior	Frequently for 6 mos after	Complete zooplankton assemblage kill 2 days after; all 42 species found before treatment found within 5 mos	Kiser et al. 1963
Patricia and Celestine lakes, Alberta, Canada	1966	0.75 mg/L 5% rotenone solution = 37.5 ppb	1 sample 2 mos prior	3 yrs after	Near complete recovery in 3 yrs	Anderson 1970
Experimental ponds, Columbia, MO	1971	0.5 and 2 mg/L 5% rotenone = 25 and 100 ppb	Biweekly for 2 mos prior, and then 14, 7, 3, 2, and 1 day pre-treatment	1, 2, 3, 7, and 14 days post-treatment and then biweekly 1 yr after	No immediate or long-term decreases in abundance or taxa observed	Houf and Campbell 1977
Lake Haugatjern, Norway	1980	0.5 mg/L 5% rotenone solution = 25 ppb	7 samples 1 yr prior	3 yrs and 4 yrs after	Small effect on zooplankton species composition and biomass	Reinertsen et al. 1990
Lake Haugatjern, Norway	1980	0.5 mg/L 5% rotenone solution = 25 ppb	Monthly, 6 mos prior	Seasonal, 2 yrs	Little change to overall benthic assemblages, except to Chironomidae fauna, *Chironomus* in particular	Koksvik and Aagaard 1984
Lake Christina, MN	1987	3 mg/L 5% rotenone solution = 150 ppb	Seasonal 2 yrs prior	Seasonal 3 yrs	Large change in zooplankton assemblages. Observed changes attributed to change in fish assemblage	Hanson and Butler 1994
Golf Course Ponds, IL	1991	0.6 mg/L 2.5% rotenone solution = 15 ppb	15 min. prior	6 mos	Full recovery in 6–8 mos	Beal and Anderson 1993
Unnamed pond, MN	1998	3 mg/L 5% rotenone solution = 150 ppb	2 samples 6 mos prior	1 yr	Large short-term effect on zooplankton, no effect after 1 yr	Melaas et al. 2001
Lake Davis, CA	2006	Estimated to be 2 mg/L 5% rotenone solution = 100 ppb	3 mos and 18 days prior	1 week, 9 mos, and 22 mos after	57% decrease in total zooplankton abundance immediately after treatment and was 58% and 61% lower after 1 and 2 yrs. Taxa richness unchanged	CA Fish and Game 2006
Orchard Ponds, New Zealand	2004	Not specified	None, design compared control and treatment ponds that were treated, 1 mo, 1 yr, and 3 yrs previously	1 sampling date–zooplankton, sweep net, and Ekman dredge samples	Zooplankton—no difference in abundance or taxa richness among treatments Benthic assemblages–no difference in taxa richness among treatments	Blakely et al. 2005

The immediate and short-term responses of aquatic invertebrates to rotenone treatments in streams have been large reductions in invertebrate abundance and taxa richness (Table 3). Aquatic insects appeared more sensitive than non-insects, and the insect groups Ephemeroptera, Plecoptera, and Trichoptera appeared more sensitive than Coleoptera and Diptera.

Aquatic invertebrate assemblage recovery following rotenone treatment varied from months to years depending on the severity of impact and often on how recovery was measured and study length. Overall invertebrate abundances generally returned to pre-treatment levels quicker than biodiversity and taxonomic composition measures. Overall assemblage abundances typically returned to pre-application levels within a few months to a year (Table 3). Recovery times for taxonomic richness and community composition measures exceeded two years in some studies (Binns 1967; Whelan 2002) and more than five years for individual species (Mangum and Madrigal 1999). Unfortunately, longer-term (two or more years of post-treatment sampling) studies of aquatic invertebrate assemblage recovery following rotenone treatments are limited (Table 3).

ANTIMYCIN EFFECTS TO INVERTEBRATES

Published studies on antimycin effects on invertebrates appear scarce compared to the occurrence of antimycin treatments. Most available literature is limited to theses and government reports, with much of it 30 to 40 years old. Of the 15 studies we located, 4 were journal publications (Kawatski 1973; Morrison 1979; Minckley and Mihalick 1981; Dinger and Marks 2007). Three were laboratory studies (Table 4), four were conducted in lentic systems (Table 5), and eight were conducted in lotic systems (Table 6).

Table 3. Field studies on the effects of rotenone on lotic invertebrates.

Location	Study year	Rotenone treatment	Pre-treatment sampling	Post-treatment sampling	Observed change in aquatic invertebrate assemblages	Citation
Robinson Creek, CA	1963	5% rotenone active, unknown concentration	None, treated/ untreated comparison	8 mos	10–50% reduction in abundance	Cook and Moore 1969
Green River, UT	1963	2.5–9.4 mg/L 5% rotenone solution = 125–470 ppb for 7 h	2 weeks prior	2 yrs after	Immediate reduction in abundance of nearly all species. Hydrosychidae (Trichoptera) recovered after 2 yrs, burrowing mayflies extirpated	Binns 1967
Strawberry River, UT	1990	3 mg/L 5% rotenone solution = 150 ppb for 48 h	1 week prior	Annually 5 yrs	54% decrease in taxa richness after 1 yr, 21% decrease in taxa richness after 5 yrs	Mangum and Madrigal 1999
Steams, Papua, New Guinea	1990	Unknown	Immediately prior	Immediately after and then up to 2 hrs	Significant declines in Dixidae and Hydropsychidae, no change in Leptophlebiide or in total abundance	Dudgeon 1990
Silver King Creek, CA	1964–1996	Treatments in 1964, 1976, 1977, 1991, and 1993. Unknown concentrations to 20 ppb for 18–24 hr in 1991 and 1993	None	Multiple times 1984 – 2006	Slight reduction in total, Ephemeroptera, Plecoptera, and Trichoptera taxa richness and change in percent dominant taxa	Trumbo et al. 2000
Manning Creek, UT	1995	0.5–1.5 mg/L 5% rotenone solution = 25–75 ppb for 12–18 hrs	1 mo prior	1 yr and 3 yrs	13% decrease in taxa richness after 3 yrs	Whelan 2002
River Ogna, Norway	2001	Unknown	Just prior	2 mos	Rapid recolonization of common taxa, a few taxa disappeared	Kjaerstad and Arnekleiv 2003
Strawberry Creek, Great Basin NP	2000	5 mg/L 5% rotenone solution = 250 ppb for 1 h and 2 mg/L 5% rotenone solution = 100 ppb for 7 hr	1 yr and 1 day prior	1 mo, 9 mo, and 1 yr after	89% reduction in total taxa richness at 1 month, 22% reduction at 1 year, 4 taxa missing at 1 year, 2 taxa missing at 3 years. 95% reduction in total abundance at 1 month, 47% reduction at 1 year	Hamilton et al. 2009
Virgin River, UT	2001–2005	11 treatments between 1988 and 2005, unknown concentrations prior to 2004. In 2004 and 2005, 3 ppm of unknown rotenone solution for 3–8 hr.	None	1 yr	Little to no change following 2004 and 2005 treatments, study complicated by lack of pre-data and > 20 yrs of rotenone treatment	Vinson and Dinger 2006

Antimycin: Laboratory studies

A summary of several laboratory studies suggests invertebrates have a wide range of sensitivity to antimycin (Table 4). Sensitivity increases with increasing water temperatures (Walker et al. 1964) and decreases at pH > 8.5 (Marking 1975). Water hardness appears to have little effect on antimycin toxicity (Lee et al. 1971). Kotila (1978) tested 18 stream invertebrate taxa to various concentrations, exposure times, and water chemisty and documented a range of tolerances with some taxa surviving 1,000 ppb over 48 hours and others suffering 50% mortality at concentrations as low as 16.9 ppb over 8 hours (Table 4).

Antimycin: Lentic habitats

Few studies of antimycin effects on invertebrates in lakes and ponds have been published in peer-reviewed journals. Initial response to antimycin appears greater for zooplankton than benthic invertebrates, where the reported impacts on assemblages have been slight (Table 5). The few reports on recovery following treatment suggest little short or long-term effects of antimycin on lentic macroinvertebrate assemblages (Snow 1974, sampling 6 years after treatment; Houf and Campbell 1977).

Antimycin: Lotic habitats

Antimycin has been used in streams since the 1970s. The extent of pre-treatment sampling varied widely across studies from none to seasonal sampling for two years prior to treatment (Dinger and Marks 2007), with the majority of studies sampling just prior to treatment (Table 6). Post-treatment sampling was similarly variable, with some studies only sampling immediately following treatment and two studies collecting samples for more than a year after treatment.

Treatment concentrations in rivers varied from < 10–100 ppb (Table 6). In general, measured effects on abundances appeared related to concentration, with significant reductions in invertebrate assemblage abundance observed at concentrations > 10–20 ppb. Similar to that observed with rotenone, zooplankton appear more sensitive than larger benthic invertebrates, small lentic invertebrates and aquatic insects appeared more sensitive than non-insects, and the insect groups Ephemeroptera, Plecoptera, and Trichoptera appeared more sensitive than Coleoptera and Diptera (Jacobi and Degan

Table 4. Summary of laboratory derived tolerances of selected aquatic invertebrate taxa to antimycin. Summarized from Kotila (1978), except for Odonata: Coenagrionidae: *Ischnura* and Cladocera (Walker et al. 1964) and Ostracoda (Kawatski 1973).

Low tolerance (0–20 ppb)	**Intermediate tolerance (20–100 ppb)**	**High tolerance (> 100 pb)**
Diplostraca Cladocera Daphniidae	Trichoptera	Trichoptera
Ostracoda	Brachycentridae: *Micrasema rusticum*	Hydropsychidae: *Diplectrona modesta*
Trichoptera	Helicopsychidae: *Helicopsyche borealis*	Lepidostomatidae: *Lepidostoma griseum*
Brachycentridae: *Brachycentrus amercanus*	Limnephilidae: *Pycnopsyche guttifer*	Plecoptera
Brachycentridae: *Brachycentrus occidentalis*	Plecoptera	Perlidae: *Perlesta placida*
Hydropsychidae: *Hydropsyche bifida*	Capniidae: *Paracapnia angulata*	Ephemeroptera
Uenoidae: *Neophylax concinnus*	Nemouridae: *Nemoura trispinosa*	Ephemerellidae: *Ephemerella*
Plecoptera	Perlidae: *Agentina capitata*	Coleoptera
Perlodidae: *Isoperla signata*	Perlidae: *Paragnetina media*	Dryopidae: *Helichus striatus*
Perlodidae: *Isoperla slossonae*	Perlodidae: *Isoperla clio*	Dytiscidae: *Agabus seriatus*
	Pteronarcyidae: *Pteronarcys pictetii*	Elmidae: *Optioservus fastiditus*
	Taeniopterygidae: *Taeniopteryx nivalis*	Elmidae: *Stenelmis crenata*
	Ephemeroptera	Psephenidae: *Psephenus herricki*
	Baetiscidae: *Baetisca lacustris*	Odonata
	Ephemerellidae: *Ephemerella invaria*	Coenagrionidae: *Argia apicalis*
	Ephemeridae: *Hexagenia limbata*	Coenagrionidae: *Ischnura* sp.
	Heptageniidae: *Leucrocuta hebe*	Corduliidae: *Neurocordulia molesta*
	Heptageniidae: *Maccafertium vicarium*	Gomphidae: *Gomphus vastus*
	Leptophlebiidae: *Leptophlebia cupida*	Megaloptera
	Potamanthidae: *Anthopotamus myops*	Corydalidae: *Nigronia serricornis*
		Diptera
		Athericidae: *Atherix variegate*
		Tipulidae: *Tipula*

Table 5. Field studies on the effects of antimycin on lentic invertebrates.

Location	**Study year**	**Antimycin treatment**	**Pre-treatment sampling**	**Post-treatment sampling**	**Observed change in aquatic invertebrate assemblages**	**Citation**
2 hatchery ponds, Delafield, WI	1963	10 ppb	Not specified	Not specified	Invertebrates were more abundant post-treatment	Walker et al. 1964
8 various ponds/ lakes, WI, WY, NE, AR, NY, and NH	1964–1966	3.12–12 ppb	Not specified	Not specified	Mortalities in 7 of 15 taxa examined, as high as 99%	Gilderhus et al. 1969
Rush Lake, WI	1967	0.5–0.75 ppb	None	Once, 6 yrs after	No gross effects 6 yrs later	Snow 1974
9 Experimental ponds, Columbia, MO	1971	20–40 ppb	Biweekly for 2 mos prior and then 14, 7, 3, 2, and 1 day pretreatment	1, 2, 3, 7, and 14 days post-treatment and then biweekly 1 yr after	No short or long term declines in abundance in 6 representative taxa observed. No change in taxa diversity.	Houf and Campbell 1977

1977; Morrison 1979; Minckley and Mihalick 1981; Moore et al. 2005; Dinger and Marks 2007; Hamilton et al. 2009).

Studies of aquatic invertebrate assemblage recovery following antimycin treatment generally reported recovery within one year (Table 6). As with rotenone treatments, invertebrate assemblage abundances returned to pre-treatment levels quicker than biodiversity and taxonomic composition measures. Longer-term studies of recovery (one or more years of post-treatment sampling) were limited to Dinger and Marks (2007) and Hamilton et al. (2009). Dinger and Marks (2007) observed shifts in species composition towards more tolerant species, but after 24 months, they concluded that there was no discernable pattern in why certain species were eradicated and others were not. Hamilton et al. (2009) reported all pre-treatment taxa were collected within 1 year post-treatment.

Table 6. Field studies on the effects of antimycin on lotic invertebrates.

Location	Study Year	Antimycin treatment	Pre-treatment sampling	Post-treatment sampling	Observed change in aquatic invertebrate assemblages	Citation
Seas Branch Creek, WI	1972	17–44 ppb	Monthly, 6 mos prior	Immediately, monthly, and bi-monthly for 2 yrs after	50–100% decrease in biomass immediately after, recovery in ~1 yr	Jacobi and Degan 1977
Ashippun River, WI	1974	7–42 ppb	None	Artificial samplers, 2 days and 5 days after	Decreases in benthic abundances observed	Kotila 1978
Ord Creek, AZ	1977	10 ppb	Immediately prior	Immediately after and 3 yrs after	Decrease in standing crop (5X numerically, 70X biomass); recovery 3 yrs later	Minckley and Mihalick 1981
Allt a' Mhuilinn, Scotland	1977	10–20 ppb	Once, 5 days prior	Once, 2 weeks after	No significant decreases	Morrison 1979
Sams Creek, TN	2001	8 ppb	Occasionally 5 yrs prior, and mo prior	Immediately after and seasonally 1 yr after	18–25% reduction in total taxa richness, recovery 1 yr after	Walker 2003, Moore et al. 2005
Snake Creek, NV	2002	8 ppb	1 yr and 1 day prior	1 mo, 9 mo, and 1 yr after	23% reduction in total taxa richness at 1 month, 10% reduction at 1 year, no taxa missing at 1 year. 10% reduction in total abundance at 1 month and 1 year	Hamilton et al. 2009
LaBarge Creek watershed, WY	2002 to 2003	10 ppb	not specified	not specified	No measurable effects	Cerreto 2004
Fossil Creek, AZ	2004	54–100 ppb	Seasonally 2 yrs prior	Seasonally 2 yrs after	Decreases in invertebrates immediately after, recovery 5 mos after	Dinger and Marks 2007

SUMMARY OF EFFECTS

For both piscicides, interpretation of the effects on invertebrate assemblages were often contradictory, with some studies reporting few treatment effects on invertebrates (e.g., rotenone—M'Gonigle and Smith 1938; Brown and Ball 1943; Ball and Hayne 1952; Zilliox and Pfeiffer 1960; Cook and Moore 1969; Houf and Campbell 1977; Finlayson et al. 2010; antimycin—Walker et al. 1964; Houf and Campbell 1977; Walker 2003; Moore et al. 2005; Hamilton et al. 2009) and other studies reporting substantial treatment impacts to invertebrates (e.g., rotenone—Davidson 1930; Cutkomp 1943; Zischkale 1952; Das and McIntosh 1961; Binns 1967; Hamilton et al. 2009; antimycin—Jacobi and Deagan 1977; Minckley and Mihalick 1981; Dinger and Marks 2007). The causes of these differences are intriguing and not entirely clear, but to us they appeared due to three factors: (1) piscicide concentration, duration, and treatment breadth; (2) aquatic invertebrate study objectives and sampling intensity; and (3) natural variation in toxicity among species and species groups.

Effects were nearly always greater at higher concentration levels. Finlayson et al. (2010) suggest a mean rotenone concentration of 25–50 ppb for < 8 h should result in complete mortality to salmonids and limited mortality to invertebrates in streams. This rotenone dosage is less than that commonly used in fish removal projects (Table 3). They also found that rotenone formulations containing secondary chemicals, such as the synergist piperonyl butoxide, contributed to toxicity to invertebrates, but not to salmonids. Additional research on the effects of secondary chemicals and refinement of minimum exposure rates is needed for more fish species so that treatment application rates are sufficient to meet project objectives, but also lessen impacts to non-target organisms.

Morphological differences among invertebrates occupying different habitats also appear to strongly influence the impact of piscicides on invertebrates. Benthic invertebrates appear less sensitive than planktonic invertebrates, smaller invertebrates appear more sensitive than larger invertebrates, and aquatic invertebrates that use gills appear more sensitive than those that acquire oxygen cutaneously, through lamellae, use respiratory pigments, or breathe atmospheric oxygen. These generalizations are similar to those described by Ling (2003) and suggest that impacts of piscicides in lotic environments may be greatest in mountain trout streams. These habitats are characterized by cold water and high oxygen levels, and are often dominated by small gilled invertebrates, namely Ephemeroptera, Plecoptera, and Trichoptera (EPT). Indeed, piscicide studies in mountain streams generally showed EPT taxa to be more susceptible than other taxonomic groups (Binns 1967; Minckley and Mihalick 1981; Mangum and Madrigal 1999; Trumbo et al. 2000; Whelan 2002; Dinger and Marks 2007; Hamilton et al. 2009). However, a rigorous evaluation among habitat types, such as high-elevation mountain streams versus low-elevation rivers has not been conducted.

Studies that tended to evaluate the effects on aquatic invertebrates as fish food availability (invertebrate assem-

PISCICIDE IMPACT STUDY DESIGN CONSIDERATIONS

Study designs to detect piscicide impacts on invertebrates will take many forms depending on the level and type of impact needing detection. While the overall question may simply be, "What is the effect of a piscicide on aquatic invertebrates?" the specifics of this question need to be addressed to develop a robust study design. Principally, will "before-after" comparisons be done based on assemblage-level measures only, such as total abundance and taxa richness; or will community composition and individual species or genera occurrences be evaluated as well?

Changes in community-level attributes are best evaluated using a BACI (Before-After-Control-Impact) study design (Underwood 1994). In BACI study designs, data are collected at control and treatment sites, both before and after the treatment. Equal numbers of control and treatment sites should be sampled for equal periods of time before and after treatment. Replication in both sites and sampling dates will increase statistical power and the ability to detect differences. For this type of study design, quantitative sampling where data are summarized as the number of individuals or taxa per a consistent sampling area is desired. The number of locations and the period of pre- and post-treatment sampling will be dependent on the heterogeneity of the system, the diversity of invertebrate assemblages, and budgets. However, we suggest that a reasonable sampling design to detect changes in community-level attributes should include four control and four treatment sites, sampled seasonally, a minimum of two years before and three years after a treatment. Statistical analysis should then follow the BACI design, using the ANOVA (Analysis of Variance) models of Underwood (1994), and insuring that the appropriate F-ratio is used to assess the impacts. For general guidance on BACI and other alternative designs (e.g., BACIPS—Before-After-Control-Impact-Paried-Series) an excellent resource is Schmitt and Osenberg (1996). Field and laboratory protocols for the collection and processing of stream invertebrate samples should follow that described in Vinson and Dinger (2008) or the Environmental Protection Agency Rapid Bioassessment of Creeks and Small Rivers single habitat (quantitative) and multi-habitat (qualitative) survey protocols (Barbour et al. 1999). Field sampling in lakes might involve collecting both zooplankton and benthic invertebrate samples. We recommend identifying invertebrates to the genus level. While species-level identifications are required to evaluate species occurrences and extirpations, this usually requires the collection of short-lived terrestrial adult stages. The effort to collect and identify adult specimens needs to be weighed against other project objectives, but in general we feel this level of detail is beyond the scope of most agencies conducting piscicide treatment assessments. Based on this design, we suggest that analysis of impacts should focus on assemblage level measures such as total abundance and taxa richness and diversity measures, and avoid assessing impacts to individual invertebrate taxa. The presence of threatened or endangered invertebrate species will obviously require different protocols for these species.

blage abundances or biomass) generally found quick recovery (e.g., M'Gonigle and Smith 1938; Brown and Ball 1943; Ball and Hayne 1952; Zilliox and Pfeiffer 1960; Walker et al. 1964; Cook and Moore 1969; Snow 1974; Houf and Campbell 1977; Trumbo et al. 2000; Moore et al. 2005); whereas studies looking at effects on invertebrate biodiversity as either in terms of individual species or species groups generally found more lingering effects (e.g., Minckley and Mihalick 1981; Koksvik and Aagaard 1984; Reinertsen et al. 1990; Beal and Anderson 1993; Mangum and Madrigal 1999; Melaas et al. 2001; Whelan 2002; Dinger and Marks 2007; Hamilton et al. 2009). These somewhat contradictory results appear due to natural variation in colonization rates among species and the amount of pre- and post-treatment sampling. In a review of 150 case studies of aquatic ecosystem recovery from disturbance, (15 of which were rotenone treatments), Niemi et al. (1990) found that recovery times of total macroinvertebrate assemblage abundances to 85% of pre-disturbance densities generally occurred in less than 18 months, whereas recovery of abundances for different taxonomic orders of insects varied widely. Recovery of Diptera abundances occurred to near 80% within 1 year, Ephemeroptera abundances to near 70% after 1 year, and Trichoptera and Plecoptera abundances recovered to only about 60% after 2 years. They found that recovery rates were influenced most by: (1) impact persistence, including changes in system productivity, habitat integrity, and persistence of the stressor; (2) organism life history, including generation time, and propensity to disperse; (3) time of year the disturbance occurred; (4) refugia presence; and (5) distance of colonization sources. They did not mention pre-disturbance densities, but the relative rarity of taxa would also likely influence their ability to repopulate an area or the ability to collect these taxa. They felt that downstream drift from unimpacted upstream areas was the critical factor in determining recovery times.

We found in general that sampling conducted a year post-treatment appeared adequate to detect impacts to assemblage measures, such as total abundance or taxa richness, but not for detecting impacts to individual taxa. The three longest duration studies to date (Mangum and Madrigal 1999; Whelan 2002; Hamilton et al. 2009) all reported the loss of several taxa, i.e., taxa found prior to treatment were not collected one year post-treatment, however many, but not all, of these taxa were found 2 to 3 years post-treatment. These studies also reported collecting a number of taxa post-treatment that were not collected pre-treatment. These results suggest two things; (1) pre- and perhaps post-treatment sampling was insufficient to adequately characterize the local fauna and (2) aquatic invertebrate assemblages are highly diverse and dynamic. Both of these factors prevent us and the authors of the original studies from conducting more rigorous analyses to determine if differences in taxa occurrences between pre- and post treatment samples were due to natural variation, sampling variation, or piscicides. No studies appear to us to have done an adequate job of describing pre-treatment assemblages with respect to the occurrence of individual taxa.

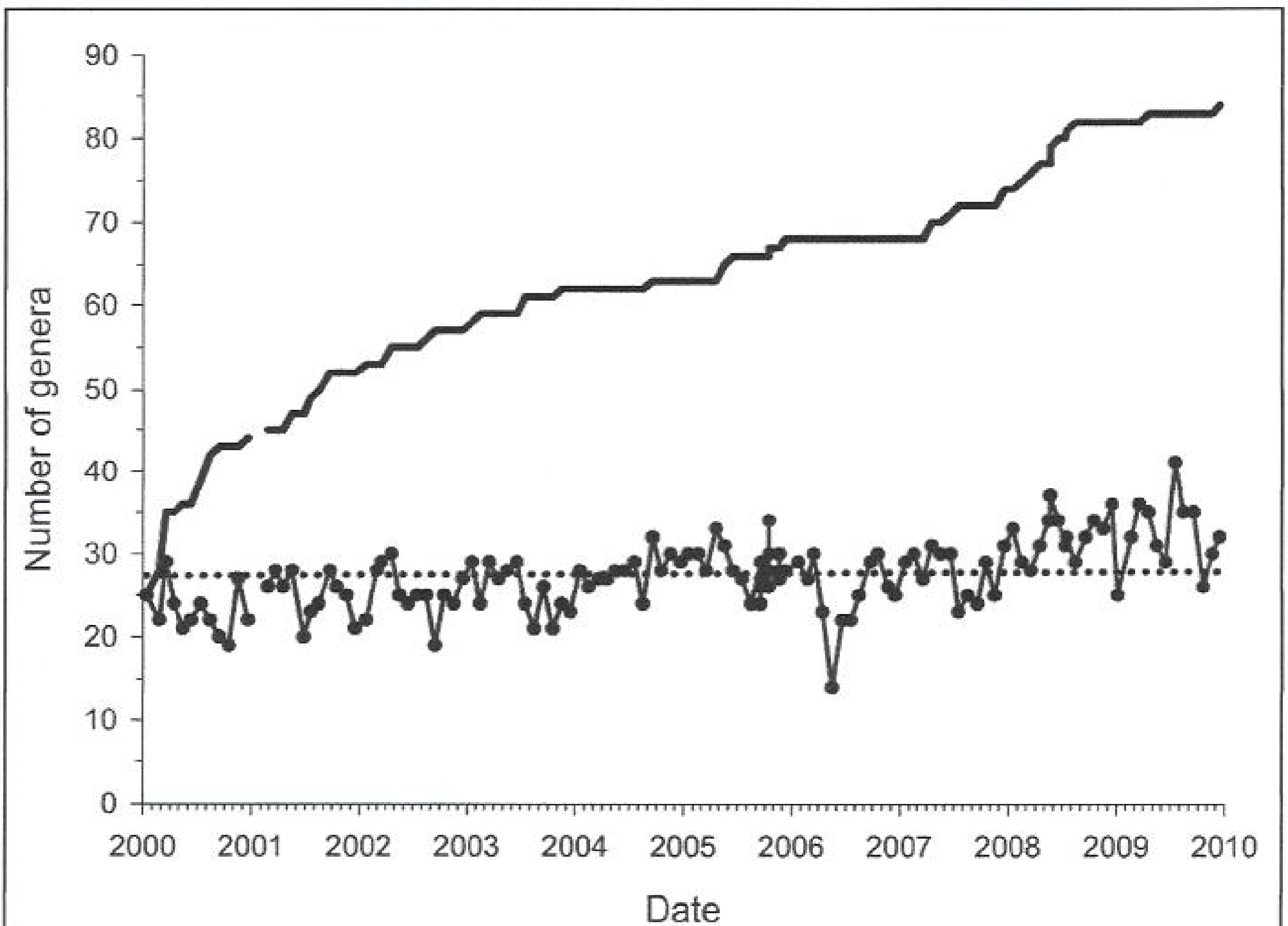
The amount of sampling necessary to provide accurate and precise measures of individual genera or species occurrences both before and after a treatment can be extensive. For stream invertebrates, the presence of large numbers of rare taxa is a common phenomenon. There have been no complete inventories of invertebrates of any body of freshwater, but several studies to date have documented that local stream reach (ca. 1 km) faunas contain hundreds to thousands of species. A total of 1,122 species have been reported from the Danube River, Austria, and 1,044 species from the Breitenbach River, Germany (Strayer 2006). In comparison, most published studies with seasonal sampling for 1 to 2 years of length seldom collect 100 genera/species and 50 to 60 genera/species is more common in a 1 km stream reach (Vinson and Hawkins 2003, M. Vinson unpublished data).

M. Vinson (unpublished data) sampled the same location on the Logan River, Cache County, Utah, monthly for 10 years. Samples were collected following standard protocols commonly used in piscicide assessment projects (field sampling methods described in Vinson and Dinger [2008] and laboratory procedures described in Vinson and Hawkins [1996]). The results of this study have shown little variation in the number of genera collected each month, but the occurrences of individual genera varies widely. To date, 84 genera have been collected at the site, but the number of individual genera collected each month averages 27.5, roughly 33% of the total genera collected in the stream reach over 10 years. On average, a new genera has been collected about every 2 months (Figure 1) and the genera accumulation curve shows little inclination for flattening out and would likely even be steeper for species-level identifications. These results, similar to that reported by Needham and Usinger (1956) and Resh (1979), led Resh (1979) to suggest that variation in aquatic invertebrate populations within a stream reach is so high that collecting data on the abundances of all but the most common taxa or the assemblage as a whole is likely beyond the scope of most assessment projects.

CONCLUSIONS

Overall, there have been too few published studies with little comparability with respect to treatment methods and invertebrate sampling efforts to allow for any sweeping statements on the overall effects of rotenone and antimycin on aquatic invertebrates in general and stream invertebrates in particular. Thus, scientists and managers must consider effects on invertebrates and the consequences on a case-by-case basis. However, recent work suggests that impacts to invertebrate assemblages can be reduced and mortality to target fish species maintained at lower concentrations than have generally been used in the past (Finlayson et al. 2010). To further reduce impacts and enhance recolonization, we recommend the following actions: (1) chemical treatments of larger drainages should stage treatments with intermediate barriers and allow time between treatments for dispersal and recolonization of invertebrates to avoid potential for cumulative impacts; (2) headwater and tributary fishless stream reaches should not be treated so they can serve as refuges for invertebrates; and (3) piscicides should be neutralized downstream of the project area to protect downstream colonization sources. We also see a need for additional laboratory toxicity tests, field studies that measure actual rotenone concentrations for the duration of the treatment so actual exposure conditions can be quantified, and longer-term (3-year pre-treatment and > 5-year post-treatment), more rigorous field evaluations of invertebrate assemblages to improve our ability to predict piscicide effects on invertebrates.

Figure 1. Monthly collections and genera accumulation curves for benthic aquatic invertebrates collected from the Logan River, Cache County, Utah between January 2000 and December 2009. Solid lines are individual monthly values (bottom) and cumulative collection (top) of unique genera. The dotted line is the long-term mean and median of 27.5 genera per sample. Five samples were collected per month in September–December 2005, three samples were collected in May 2008, and two in July 2008. No sample was collected in January 2001.



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