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Research Article

Evaluating the piscicide rotenone as an option for eradication of invasive Mozambique tilapia in a Hawaiian brackish-water wetland complex

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Abstract

Mozambique tilapia *Oreochromis mossambicus* were recently discovered in 'Aimakapā Fishpond, a 12-hectare brackish-water wetland complex in Kaloko-Honokōhau National Historical Park, on the Island of Hawai'i. As a possible eradication method, we evaluated rotenone, a natural piscicide used in fish management and the active ingredient in plants traditionally used by indigenous Hawaiians for capturing fish. To assess rotenone's efficacy in killing tilapia and effects on non-target species, laboratory toxicity tests involved exposing organisms to various concentrations of liquid CFT Legumine (5% rotenone) in static trials of 48-h to 72-h duration. Test organisms included: Mozambique tilapia, non-native guppy *Poecilia reticulata*, the non-native odonate Rambur's forktail *Ischnura ramburii*, native feeble shrimp *Palaemon debilis*, and native 'ōpae'ula shrimp *Halocaridina rubra*. All organisms and water used in tests were obtained from 'Aimakapā (12.6–12.7 ppt salinity), or, for *H. rubra*, an anchialine pool (15.0–15.2 ppt salinity). Survival analyses indicated CFT Legumine concentrations ≥3 ppm (>0.15 mg/L rotenone) achieved 100% mortality of tilapia and 93% of guppies within 24 h, with most tilapia killed by 6 h and most guppies by 2 h. Little or no mortality was observed among invertebrate exposed to 1 to 5 mg/L CFT Legumine: 0% mortality for 'ōpae'ula shrimp, 4% for feeble shrimp; and 16% for odonate larvae. The 48 h LC_{50} values for Mozambique tilapia and guppy were 0.06 and 0.11 mg/L rotenone, respectively. Results demonstrate rotenone's potential for non-native fish eradication in brackish-water habitats, with benefit of low mortality to certain macro-invertebrates. High rotenone tolerance displayed by 'ōpae'ula shrimp is noteworthy. Invasive fish are common in anchialine pools, threatening existence of shrimp and other invertebrate fauna. Although rotenone's effects on freshwater organisms have been well studied, our research represents one of only a few controlled laboratory experiments quantitatively assessing rotenone tolerance of brackish or marine fauna.

Key words: anchialine pools, brackish-water environments, Cichlidae, Hawaiian fishponds, invasive fish eradication, Pacific Islands

Introduction

As many as 52 different introduced non-native fish species have wild reproducing populations in the Hawaiian Archipelago (Maciolek 1984; Yamamoto and Tagawa 2000; Mundy 2005; Carlton and Eldredge 2009; See et al. 2009). Moreover, certain introduced fish taxa are broadly euryhaline, some capable of surviving and even reproducing in fresh, brackish, and marine environments. In many areas, non-native fish are abundant and in some locales their biomass and numbers exceeds those of the native fish fauna (Yamamoto and Tagawa 2000). The ecological impacts from such an array of introduced fishes on Hawaii's ecosystems and native organisms are diverse, complex, often not readily observed, and frequently unpredictable (Maciolek 1984; Englund 1999; Eldredge 2000; Yamamoto and Tagawa 2000; Englund and Polhemus 2001; Font 2003; Capps et al. 2009; Dalton et al. 2013). Given the actual and potential threats posed by introduced fishes, natural resource managers and conservation biologists have reason for concern, particularly since there are few options currently available for eradicating or controlling these invaders (Kolar et al. 2010; Nico and Walsh 2011).

The Mozambique tilapia *Oreochromis mossambicus* (Peters, 1852), a highly invasive euryhaline fish, was recently discovered in

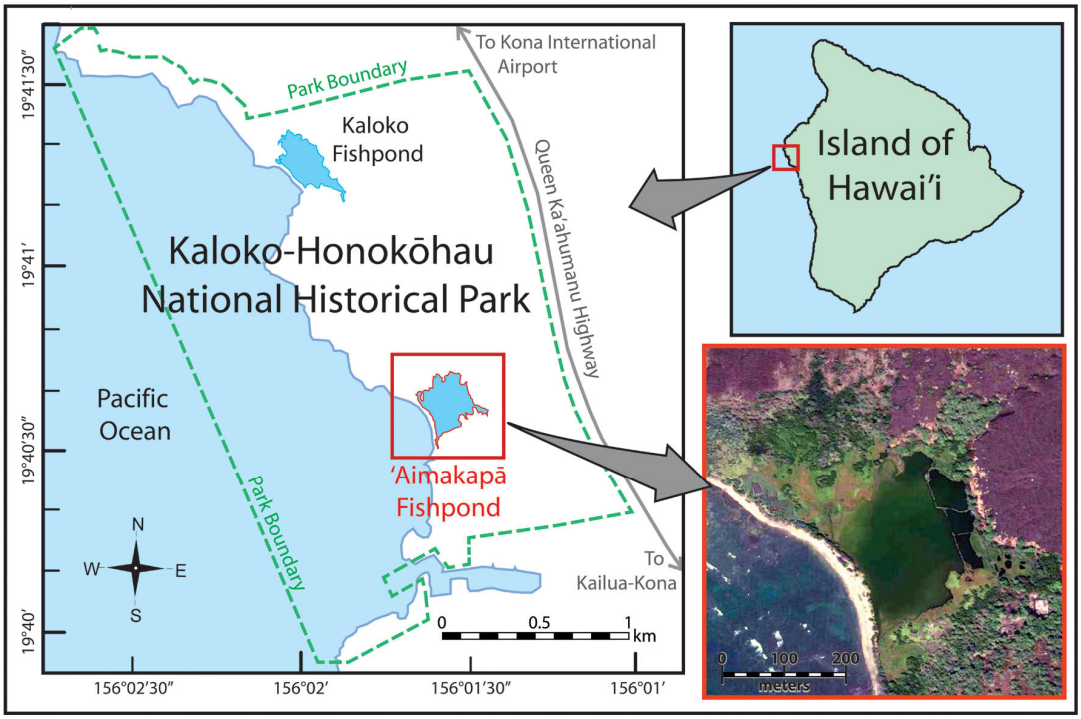

Figure 1. Map showing location of Kaloko-Honokōhau National Historical Park (KAHO) on the Island of Hawai'i and a satellite photographic image of the 'Aimakapā Fishpond wetland complex and adjacent habitats.

'Aimakapā Fishpond (hereafter 'Aimakapā), a brackish-water wetland complex in Kaloko-Honokōhau National Historical Park (KAHO) on the Island of Hawai'i (Figure 1) (MacKenzie and Bruland 2012). Its occurrence in 'Aimakapā represents the first confirmed record of a tilapia in the wetland and the only known established tilapia population in KAHO. Predation by Mozambique tilapia is likely having a significant adverse effect on the invertebrate community. Mozambique tilapia are established in inland and coastal waters of many Pacific Islands (Lobel 1980; Maciolek 1984; Lever 1996). By competetion for resources and predation on the young or fry of other fishes, tilapia are frequently blamed for localized decreases of commercially valuable native fishes, such as milkfish *Chanos chanos* (Forsskål, 1775), striped mullet *Mugil cephalus* Linnaeus, 1758, and bonefish *Albula vulpes* (Linnaeus, 1758) (Lobel 1980; Ranoemihardjo 1981; Nelson and Eldredge 1991; Spennemann 2002; Fortes 2005). There is reasonable likelihood that the tilapia inhabiting 'Aimakapā will eventually disperse into adjacent habitats, including nearby anchialine pools and coastal marine waters. Anchialine pools are mixo-haline coastal habitats without surface connection to the ocean, but show tidal fluctuations (Holthuis 1973). If tilapia do invade KAHO's anchialine pools, they would threaten the existence of several native aquatic invertebrates already imperiled or of special ecological significance. Local endemic native invertebrates perhaps most at risk are the damselfly *Megalagrion xanthomelas* (Selys-Longchamps, 1876), a snail of the genus *Neritilia*, and the shrimp *Halocaridina rubra* Holthuis, 1963 (Polhemus 1996; USNPS 2013).

Tilapia eradication is being investigated in an effort to restore natural communities and functions of the 'Aimakapā wetland complex. Among options being assessed is rotenone, a natural piscicide long used in fish management (Ling 2003; McClay 2005; Finlayson et al. 2010a). Rotenoid compounds are also the active ingredient in plants traditionally used by indigenous Hawaiians and other Pacific Islanders for capturing fish (Barrau 1955; Rickard and Cox 1986; Armstrong et al. 2011). Rotenone has been used with limited success in the past to remove invasive fish from natural anchialine pools in Hawaii (Nico and Walsh 2011). To date, there are no published experimental or quantitative data on the effects of rotenone on

Hawaii's aquatic invertebrates. Review of the literature revealed that few laboratory experiments have ever been conducted to assess quantitatively rotenone's lethal effects on brackish-water or marine fishes and invertebrates.

This paper presents results of laboratory experiments conducted to determine if rotenone is a viable option for eradication of Mozambique tilapia established in 'Aimakapā and assess if the chemical would be harmful to certain non-target aquatic organisms present in or near the wetland. Our objectives were to: 1) determine the minimum concentration of rotenone necessary to achieve 100% mortality of Mozambique tilapia; 2) assess the effect of various rotenone concentrations on non-target species present in 'Aimakapā, including non-native guppy *Poecilia reticulata* Peters, 1859, larvae of the non-native odonate Rambur's forktail *Ischnura ramburii* (Selys, 1850) (an experimental surrogate for native imperiled odonate species), and native feeble shrimp *Palaemon debilis* Dana, 1852; and 3) assess the effect of rotenone on 'ōpae'ula shrimp *Halocaridina rubra*, a Hawaiian native endemic that inhabits anchialine pools in KAHO and other parts of Hawai'i. All rotenone bioassay experiments used brackish water from sites of capture, either 'Aimakapā, or, for *H. rubra*, a nearby anchialine pool.

Methods

Study area

KAHO is located along Hawai'i's western shoreline (19°41′ N, 156°01′30″W; Figure 1) and managed by the US National Park Service for the preservation and protection of cultural and anthropological resources and to preserve and enhance other natural resources (Parrish et al. 1990). The Park covers approximately 470 ha, consisting of about 230 ha of land (including inland waters) and 240 ha of adjacent marine waters. KAHO's most notable inland aquatic habitats are two large, historically important fishpond-wetland complexes known as 'Aimakapā and Kaloko, and the more than 130 small anchialine pools (Bienfang et al. 2011). 'Aimakapā covers ~12 hectares, consisting of a 4.7-ha shallow (<1.5 m deep at high tide), open-water "pond" fringed by about 7.6 ha of mostly emergent marsh. It is separated from the ocean by a narrow sand berm 3-m high and 32-m wide (Vitousek et al. 2009). 'Aimakapā's water is brackish, consistently about 12 ppt salinity during current study. Water levels fluctuate throughout the day in delayed response to changing ocean tides. Salinity and water levels result from the site's highly permeable volcanic rock substrate, which allows passage of a mix of fresh and salt water from underground sources (Oki et al. 1999).

'Aimakapā is of major ecological importance as a refuge for native and migratory wetland birds and provides foraging and nesting habitat for two federally-listed endangered birds endemic to Hawai'i, the Hawaiian stilt or ae'o (*Himantopus mexicanus knudseni* Stejneger, 1887) and Hawaiian coot or 'alae ke'oke'o (*Fulica alai* Peale, 1848) (Morin 1994, 1998; USFWS 2011). In contrast, 'Aimakapā is of low ecological value for native fishes due to the long absence of a surface connection to the ocean making habitat unavailable as a spawning and nursery area to most marine/estuarine species. During 2012–2013, we intensively sampled 'Aimakapā' and found Mozambique tilapia and guppy to be widespread and abundant. Only three native fishes were observed and none were common: milkfish, bluefin trevally *Caranx melampygus* Cuvier in Cuvier and Valenciennes, 1833 (not previously recorded for 'Aimakapā), and a mullet, most likely striped mullet. The few other native fishes previously reported have either already disappeared from 'Aimakapā, presumably through attrition, or are so rare that they now go undetected.

Rotenone experiments

Rotenone experiments were conducted during two separate sessions, session 1 in June 2012 and session 2 in July 2012 at temporary laboratories in the Kona coast area of Hawai'i Island outside KAHO. Laboratories were without artificial climate control, freely exchanged air with the outside, and exposed to natural cycles of photoperiod, the same 24-h diel cycle experienced by 'Aimakapā, about 13 hours light and 11 hours dark. All experiments consisted of plunge-type acute (abrupt) tolerance testing under static, non-renewal conditions (i.e., test water not replaced during experimental period) and, except for a few modifications, followed protocols of Schofield and Nico (2007). Acute rotenone testing (versus gradual rotenone increases) in a static setting better simulates how fisheries biologists typically apply rotenone for fish eradication in lacustrine habitats (Finlayson et al. 2000). All water used to transport live animals from field to laboratory, for holding animals, and for preparation of the different treatment concentrations (i.e., test waters) was brackish water obtained from sites where test

Table 1. Summary information on Mozambique tilapia and other taxa from ‘Aimakapā and vicinity tested during rotenone exposure experiments (5 different rotenone concentrations and a control). Included are numbers of individuals tested, numbers of replicates for each treatment, ranges and means of body lengths and wet weights, volume of test solution per treatment chamber, and calculated loading factors (i.e., ratio of organism mass to test water volume). Body lengths for fish represent standard length (SL); body lengths for invertebrates represent total body length (see text). Abbreviations: N = total number of individuals tested; SD = Standard Deviation. Loading factor values > 1 g/L are given in bold.

Taxa tested	N	Replicates per treatment	Body length (mm) Range [mean ± SD]	Wet Weight (g) Range [mean ± SD]	Treatment chamber liquid volume (L)	Loading factor (g/L) range [mean]
Experiment session 1						
Mozambique tilapia *O. mossambicus*						
All	60	10	27-152 [80 ± 33.3]	0.6-81.0 [22.4 ± 21.8]	7 & 1	0.60-**11.57** [**5.14**]
Large: >70 mm SL	30	5	74-152 [109 ± 18.9]	10.6-81.0 [40.3 ± 17.4]	7	**1.51-11.57** [**5.75**]
Small: <70 mm SL	30	5	27-64 [50 ± 9.5]	0.6-8.1 [4.5 ± 2.1]	1	0.60-**8.10** [**4.50**]
Guppy *Poecilia reticulata*	30	5	15-22 [18.6 ± 1.9]	0.07-0.2 [0.14 ± 0.04]	0.2	0.35-1.00 [0.65]
Odonate larvae *Ischnura ramburii*	30	5	13-22 [18.5 ± 2.3]	0.014-0.044 [0.029 ± 0.010]	0.2	0.07-0.22 [0.15]
Feeble shrimp *Palaemon debilis*	30	5	33-44 [39.1 ± 2.8]	0.28-0.41 [0.35 ± 0.03]	0.2	**1.40-2.05** [**1.75**]
Experiment session 2						
‘Ōpae’ula shrimp *Halocaridina rubra*	60	10	7.5-11.5 [9.1 ± 1.1]	0.008-0.023 [0.013 ± 0.004]	0.2	0.04-0.12 [0.07]

animals were collected, either ‘Aimakapā Fishpond (for session 1 experiments) or an anchialine pool (for session 2 experiments).

The experimental design for each session was randomized with each species exposed to six different treatments or rotenone concentrations: 0 (control), 1, 2, 3, 4, and 5 parts per million (ppm) of liquid CFT Legumine™ (5% rotenone), corresponding to 0, 0.05, 0.10, 0.15, 0.20, and 0.25 mg/L active rotenone ingredient, respectively. Depending on taxa, there were 5 or 10 replicates per treatment group for each species tested, with each replicate having one organism per test chamber. Table 1 provides a summary of the numbers of individuals of each species tested, numbers of replicates for each exposure treatment, ranges and means of body lengths and wet weights, treatment chamber volumes, and calculated loading factors (i.e., ratio of organism mass to test water volume).

CFT Legumine

We chose CFT Legumine™® (hereafter, CFT Legumine), manufactured by Prentiss, Inc., for testing because it is a widely-used, recent formulation specifically designed to reduce or eliminate a number of hydrocarbon compounds without any synergists. Consequently, CFT Legumine has fewer environmental impacts compared with earlier commercial rotenone formulations, with reduced risks for applicators, public health, and terrestrial and many aquatic species (McClay 2005; Fisher 2007; Finlayson et al. 2010b). The formulation is also difficult for fish to detect, increasing the likelihood of successful fish eradication. CFT Legumine is comprised of five major constituents with the following average concentrations: rotenone (5.12%), rotenolone (0.718%), methyl pyrrolidone (MP; 9.8%), diethylene glycol monethyl ether (DEGEE; 61.1%), and Fennedefo 99 (17.1%) (Fisher 2007; Vasquez et al. 2012). Rotenone is the active ingredient and rotenolone is a degradation product. MP, DEGEE, and Fennedefo 99 are inert carrier components used as solvents and surfactants to aid in the dissolution of rotenone, all are highly soluble in water, do not tend to bind to sediment particles, do not readily volatilize from surface waters, and reportedly degrade in place via microbes or sunlight (Vasquez et al. 2012). CFT Legumine contains trace amounts (<1%) of inert benzenes of low volatility that rapidly degrade

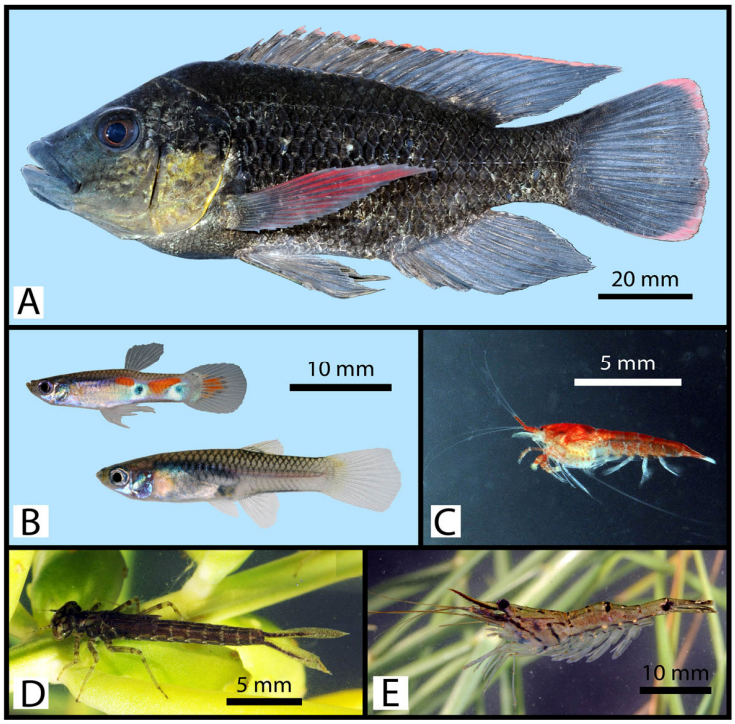

Figure 2. Photographs of representative individuals of the five species from brackish-water sites of KAHO used in rotenone-exposure experiments: (A) Mozambique tilapia *Oreochromis mossambicus*, non-native, adult male, 112 mm SL; (B) guppy *Poecilia reticulata*, non-native, adult male (above) and female, 16 and 23 mm SL, respectively; (C) ʻōpaeʼula shrimp *Halocaridina rubra*, native, adult, ~10 mm TL; (D) Rambur's forktail *Ischnura ramburii*, non-native, odonate larval stage, ~20 mm TL; and (E) feeble shrimp *Palaemon debilis*, native, adult, ~40 mm TL. All organisms were from ʻAimakapā Fishpond wetland complex, except ʻōpaeʼula shrimp which were taken from a nearby anchialine pool (photographs by H.L. Jelks).

through photolytic and biological mechanisms (Fisher 2007). We chose liquid CFT Legumine for tests rather than powder because the liquid would be easier to apply and evenly disperse in a site such as ʻAimakapā where there are mats of emergent aquatic vegetation. Because it is a liquid, we report CFT Legumine concentrations in "ppm". However, when referring to active rotenone concentrations and results from other studies that used powdered rotenone, we use "mg/L" units since rotenone is a solid. The CFT Legumine (5% rotenone) used in experiments was obtained direct from the manufacturer in a 1-L amber-colored bottle labeled 30 April 2012 that was kept cool and in the dark prior to use to prevent rotenone photodegradation.

Test animals

Five different species were used in rotenone-exposure tests: Mozambique tilapia, two other non-native taxa (one fish and one odonate insect), and two native shrimp species (Figure 2, Table 1). All individuals tested were wild-caught, obtained in June-July 2012 within a few days prior to experiments. Four of the five species were from ʻAimakapā; the exception was ʻōpaeʼula shrimp, which were collected in a nearby brackish-water anchialine pool. Basic information on each of the five test species follows:

1) Mozambique tilapia *Oreochromis mossambicus*, family Cichlidae (Figure 2A)—Tilapia were captured with traps, cast nets, and lift nets from open and vegetated areas of ʻAimakapā. Captured tilapia were separated into two different size groups and these were divided equally among treatments to insure that each treatment included a wide range of sizes (Table 1). There were no significant differences in standard length (SL) or mass of the tilapia between rotenone treatments (Kruskal-Wallis one-way analysis of variance by ranks, $df = 5, 60$; H_{SL} (corrected for ties) = 0.8158, $P = 0.9760$; mass: H_{mass} (corrected for ties) = 0.7563, $P = 0.9797$).

2) Guppy, *Poecilia reticulata* family Poeciliidae (Figure 2B)—This small, non-native livebearer

is abundant in near-shore shallows and marsh pools of ‘Aimakapā. Tests were conducted with adult males and females collected using hand nets, traps, and lift nets.

3) Rambur’s forktail *Ischnura ramburii*, order Odonata, family Coenagrionidae (Figure 2D)—This non-native species is common in the ‘Aimakapā complex and nearby areas and was chosen, in part, to serve as a surrogate for native odonate species which are now rare in KAHO and other parts of the island. Tests were conducted with aquatic larvae collected with small-meshed dip nets.

4) Feeble shrimp *Palaemon debilis*, family Palaemonidae (Figure 2E)—This native species, locally known as ‘ōpae huna, is common and widespread in brackish-water habitats in Hawai’i. Feeble shrimp used in experiments were collected with hand nets and traps.

5) ‘Ōpae‘ula shrimp *Halocaridina rubra*, family Atyidae (Figure 2C)—This tiny shrimp is endemic to the Hawaiian Islands and considered a keystone herbivorous species in anchialine pool systems (Bailey-Brock and Brock 1993; Dalton et al. 2013). Specimens used in experiments were collected with small-meshed hand nets from an anchialine pool in KAHO just north of ‘Aimakapā.

Transport, handling, and measurements

In the field, captured animals intended for possible use in experiments were separated by species, placed in appropriate-sized containers with water from capture site, and then immediately transported to the laboratory, a foot and vehicle journey of about 1 hour duration. Upon arrival at the laboratory, species were kept separate and transferred to holding tanks containing brackish water obtained from capture sites and supplied with aeration under static conditions. Holding tanks for tilapia consisted of two blue plastic "kiddy-pools", 1 m in diameter and 20 cm deep, covered with fine-mesh nets to prevent escape. Holding tanks for guppy and invertebrates consisted of an assortment of plastic tubs containing 1 to 15 L water, depending on numbers and sizes of organisms.

To reduce possible handling stress of test animals, precise measurements of body length and wet weight of individuals of each species was conducted after their death or, for test survivors, following experiment completion. Length was measured with digital dial calipers or a measuring board. For tilapia and guppies, both SL and total length (TL) were recorded. TL of odonate larvae was determined by measuring distance from anterior edge of head to posterior edge of caudal gills. TL of feeble shrimp and ‘ōpae’ula shrimp was determined by measuring distance from posterior margin of telson to anterior tip of rostrum or to most distal part of eye, whichever distance was greater. Length of ‘ōpae’ula shrimp was measured to nearest 0.5 mm; lengths of all other animals to nearest mm. Body wet weight of tilapia was determined to nearest 0.1 g using a balance accurate to 0.1 g. Body wet weights of guppies and feeble shrimp were to nearest 0.01 g and those of odonate larvae and ‘ōpae’ula shrimp to nearest 0.001 g using a Mettler Toledo AG204 DeltaRange analytical balance.

Preparation of treatment solutions

For both experimental sessions 1 and 2, the different rotenone treatment concentration mixtures were prepared within one hour of experiment initiation by serial dilutions of the CFT Legumine stock solution. The brackish-water used for creating control and rotenone test solutions was obtained from field sites 1–3 days prior to initiation of experiments. The water was temporarily stockpiled in the laboratory in clean plastic holding tanks and then, prior to mixing with CFT Legumine, was filtered through a fine-mesh net to remove large particulate matter.

Salinity and conductivity of water in the field and in the laboratory were measured with a YSI® meter model 85. Water pH was measured with a Hach Test Kit model 17-F (bromthymol blue pH range of 5.5 to 8.5). In the laboratory, water temperature was recorded hourly with HOBO Pendant® UA-002-64 data loggers throughout experimental periods: during experimental session 1, two temperature loggers were used, each submerged in randomly chosen test chambers, including one large and one of intermediate size; during experiment session 2, a single logger was submerged in a small water-filled plastic container placed next to other test chambers. The laboratories of sessions 1 and 2 were located at slightly higher elevations than ‘Aimakapā and KAHO, therefore air temperatures tended to be cooler. To document differences in water temperatures in the laboratory and field, temperature loggers were also deployed in ‘Aimakapā and in the ‘ōpae’ula shrimp anchialine pool over periods that included the time spanning the laboratory experiments.

Test chambers

Experimental chambers consisted of clear plastic containers manufactured by Sterilite®, Bella™ and Ziploc®, all newly purchased locally. Containers were thoroughly cleaned and rinsed with tap water prior to use and each container given a unique color and alpha-numeric coded label. For all experiments, animals tested were placed one individual per experimental chamber so as to avoid pseudo-replication. Due to differences among the different groups of organisms being tested, three different sizes of containers were used as test chambers (Table 1): 1) large containers (rectangular 10-L plastic bins, 40 cm long × 25 cm wide × 15 cm high) filled to a depth of about 8.5 cm, for tests with large tilapia; 2) intermediate-sized containers (12.8 cm × 12.8 cm × 9.3 cm high) filled to the 4.2 cm depth mark, for tests with small tilapia; and 3) small containers (circular, 9.5 cm diameter × 5.4 cm high) filled to the 3.8 cm depth mark for tests with guppies, odonate larvae, feeble shrimp, and ‘ōpae’ula shrimp. Each test chamber container included a snap-on lid that prevented escape. Chambers were not artificially aerated, but lids were drilled with several small holes, about 5-mm diameter, to increase air circulation. For each experimental session, all holding tanks and control and experimental chambers were in close proximity, in a single well-ventilated space and exposed to the same diel temperature fluctuations and photoperiods during pre-trial and test periods.

Rotenone trials

Animals chosen for experiments included only those that appeared healthy (e.g., normal swimming, no obvious physical deformities or evidence of infection). For each of the five taxa tested, individuals were randomly assigned to treatment chambers with aid of a random numbers table. For Mozambique tilapia, the two size groups were randomly divided among different treatments so that each of the six treatments included 5 small- and 5 large-sized individuals. Several minutes before the experiment was initiated, each test chamber was filled with the appropriate volume of test water. Organisms were then gently netted and rapidly transferred from holding tank directly to test chambers already containing test water, at which point rotenone exposure testing began. Experimental animals were not provided food during tests. Individual animals of each test species were kept in their test chamber until death, or, if still alive, for a short period after termination (endpoint) of the experiment. Dying organisms tended to lose equilibrium and lay on bottom. Death was confirmed if individual remained immobile (e.g., fish ceased all opercular movements) for several minutes and did not respond to prodding.

Experiment session 1. Four of the five species were tested during experiment session 1 (Table 1): Mozambique tilapia, guppy, the odonate Rambur’s forktail, and feeble shrimp. Water for session 1 tests was obtained from the main pond of ‘Aimakapā on 10–11 June 2012, test animals were collected from ‘Aimakapā on 12–13 June 2012, and rotenone experiments were conducted 14–16 June 2012. The experimental endpoint for tests was 48 h. Organism condition and mortality was assessed for all treatment groups every 2 h for the first 12 h, every 6 h from hours 12 to 48.

Water obtained from ‘Aimakapā and used in session 1 experiments had a salinity ranging from 12.6 to 12.7 ppt, conductivity of 12.2 mS, and a pH of 7.6. Hourly water temperatures recorded in two of the test chambers during the 48-h experimental period of session 1 ranged from 21.1 to 26.4°C (mean = 24.0°C). Hourly surface water temperatures recorded in the open water of ‘Aimakapā over a 4-day period (13–17 June 2012), hours that included the period when session 1 laboratory experiments were being run, ranged from 26.3 to 34.4°C (mean = 29.8°C).

Experiment session 2. The objective of session 2 was to determine the rotenone tolerance of ‘ōpae’ula shrimp (Table 1). Water used in session 2 tests was obtained from the anchialine pool site on 21 July 2012, the 60 ‘ōpae’ula shrimp were collected from the same pool on 24 July 2012, and rotenone experiments were conducted 25–28 July 2012. Exposure test procedures followed those of experiment session 1, except that ‘ōpae’ula shrimp trials were allowed to run to an endpoint of 72 h to better assess their long-term survival. Organism condition and mortality were assessed for all treatment groups every 2 h for the first 12 h, every 6 h from hours 12 to 48, and at hours 60 and 72.

The water used in experiments had a salinity ranging from 15.0 to 15.2 ppt, conductivity 23.30 to 24.74 mS, and a pH of 7.3. Hourly water temperatures in a small plastic container over the 72-h experimental period ranged from 18.5 to 25.9°C (mean = 22.8°C). Hourly water temperatures (22–27 July 2012) in the anchialine pool where the ‘ōpae’ula shrimp were captured ranged from 21.1 to 33.7°C (mean = 24.7°C).

Loading

In fish eradication projects, rotenone is applied to waterbodies with the objective of achieving a lethal concentration. Total mass of the wild fish population targeted for removal relative to water volume in the environment is not considered in determining the rotenone concentration. In contrast, guidelines for conducting acute toxicity tests in laboratories address the issue of loading, the mass of individual fish or invertebrates relative to the volume of solution in the test chamber. Guidelines of the American Society for Testing and Materials (ASTM) indicates loading be limited to ensure that: 1) the concentrations of dissolved oxygen and test material do not fall below acceptable levels, 2) concentrations of metabolic products do not exceed acceptable levels; and 3) the test organisms are not stressed because of aggression or crowding (ASTM 2007). However, general recommendations vary on maximum allowable loading, partly depending on temperatures, and range from about 0.5 g to 1 g of animal per liter of test solution (ASTM 2007; Finlayson et al. 2012). During the current study, the problem of crowding was avoided because test chambers in all experiments only contained a single fish or invertebrate. Based on high survival of controls across the different species tested and observations of their behavior, it was concluded that dissolved oxygen and metabolites were not an issue. ASTM guidelines address the possibility that metabolites may accumulate, increasing the risk of death and making a tested chemical appear more toxic than it really is. In contrast, fish metabolism may actually degrade rotenone with the result that the chemical would falsely appear less toxic, especially in situations where loading is not taken into consideration.

A loading value of 1 g organism/L test water is not difficult to achieve when testing very small organisms, but becomes increasingly difficult with larger animals because of the need for larger test chambers and larger volumes of test water. In the present case, the largest tilapia tested weighed 81 g and would have necessitated a test chamber holding 81 L or more of test water. Because the current study used water from ‘Aimakapā in most experiments, which required hauling large volumes of water over land by hand and vehicle, it was impractical, given logistical constraints, to haul a total of 1,380 L of ‘Aimakapā water to the laboratory for the 60 tilapia tested so that each was under the loading recommendation. Consequently, loading in most test chambers containing Mozambique tilapia and feeble shrimp exceeded 1 g organism/L (Table 1). To assess whether tilapia survival results were influenced by variations in loading among and within treatments, a multiple linear regression analysis was performed to describe and assess the relationship of tilapia survival time (dependent variable) as a function of two predictor variables: loading (g of test animal/L of test water) and rotenone concentration (mg/L of 5% rotenone).

Data analysis

Survival data of test species were plotted and statistically analyzed with a Kaplan-Meier product limit estimator (Kaplan and Meier 1958). Log-Rank (Mantel-Cox) tests were performed to compare resulting Kaplan-Meier survivorship curves, within and among experiment treatments, and evaluate whether or not survivorship curves were statistically equivalent. The Log-Rank test is used to test the null hypothesis that there is no difference between populations in the probability of an event (i.e., death) at any time point (Bland and Altman 2004). Kaplan-Meier and Log-Rank tests were performed with WinSTAT® version 2012.1 and survivorship curves graphed with GraphPad Prism® version 5.03. To control for false positives in the multiple tests (curves considered different that are actually equivalent), the Bonferroni correction was used to adjust the overall significance level P=0.05. GraphPad InStat® version 3.06 was used to analyze animal length and weight data and perform multiple regressions to evaluate relationships between survival and loading values (Table 1).

Kaplan-Meier survival analysis was chosen as our main statistical method and for illustration of results rather than median lethal dose (LC_{50}) determinations. Advantages of the approach are highlighted in the literature (Newman and Aplin 1992; Newman and Dixon 1996; Shimps et al. 2005; Zhao and Newman 2004; Newman 2013). In general, compared to concentration-effect methods, a survival analysis approach increases statistical power because more data are collected, with time-to-effect (i.e., death) of every individual noted. It also allows for description of survivorship patterns at all exposure times instead of only one exposure time. Consequently, Kaplan-Meier survival analysis is more meaningful than the more common calculation of dose required to kill fifty percent of the population in a discrete time interval (LD_{50} or LC_{50}), especially with an eradication project in which the goal is to remove all individuals of a target species (M.C. Newman, pers. comm. 2014).

Two previously published laboratory studies on rotenone toxicity to Mozambique tilapia, each conducted in fresh water, used probit analysis to report results in terms of LC_{50} (Rowe-Rowe 1971; Cruz-Lucierda 1992). Consequently, for comparative purposes, LC_{50} values were also derived using probit analysis at 2, 6, 12, 24, and 48-h time intervals for the Mozambique tilapia concentration-mortality data produced by the acute mortality experiments of current study. In this study, LC_{50} values are expressed in terms of mg of rotenone per liter of test solution. In comparing any two resulting LC_{50} values, the higher represents greater tolerance, meaning that it takes a relatively higher concentration of the chemical to cause a response (i.e., mortality). Confidence intervals (95%) for LC_{50} values were obtained using GraphPad Prism® version 5.03.

Results

Laboratory tests showed that the rotenone formulation CFT Legumine at moderately high concentrations (≥3 ppm) was effective in killing Mozambique tilapia and guppies; in contrast, the chemical had little or no effect on the three aquatic invertebrate taxa tested (Figures 3–4).

Experiment session 1

Mozambique tilapia

Statistical analysis of the Kaplan-Meier survivorship curves for Mozambique tilapia indicated a significant difference among the six different treatments (Log-Rank test, χ^2=55.78, df=5, P<0.0001). CFT Legumine concentrations 3 ppm achieved 100% mortality of Mozambique tilapia by 24 h, with most individuals (28 of 30 or 93%) in these three treatments killed by 6 h (Figure 3(A)). Of 20 individuals exposed to 1–2 ppm CFT Legumine, 9 (45%), including 5 adults and 4 juveniles, survived to the 48-h experimental endpoint. Control survival was 80% (8 of 10 individuals); the two mortalities included an adult and a juvenile found dead at 18 h and 30 h, respectively.

Pairwise comparisons of Kaplan-Meier curves indicated that survival among the three highest concentrations tested (3, 4, and 5 ppm) were not significantly different (Table 2). In contrast, pairwise comparisons showed that survival in the control group was significantly greater than that of all groups exposed to rotenone except for the 1 ppm treatment group (Table 2). Moribund tilapias exposed to higher concentrations of rotenone were markedly sluggish and generally remained inactive. Prior to death, most tilapia laid on their side; however, a few individuals that lost equilibrium later recovered to an upright position and survived until the experimental endpoint.

Guppy

Guppy survival varied significantly among the six different treatments (Log-Rank test, χ^2=21.91, df=5, P=0.0005). CFT Legumine concentrations ≥3 ppm achieved 93% mortality of guppies by 2 h (14 of 15 individuals) (Figure 3(B)). The only survivor was a female fish exposed to 3 ppm that survived beyond the 48-h experimental endpoint. Of 2 males and 8 females exposed to 1–2 ppm CFT Legumine, 9 (90%) survived beyond the 48-h experimental endpoint. Of five guppies in the control group, the lone mortality was a female found dead at the 36 h mark. Although the designated experimental endpoint was 48 h, all of the survivors were still alive in test chambers at 76 h. Guppies are live-bearing fish and during the experiment two females, both in the control group, gave birth to young.

Odonate larvae

Larvae of the non-native Rambur's forktail exhibited high tolerance to rotenone exposure (Figure 3(C)). There was no significant difference in survival among the different treatments (Log-Rank test, χ^2=4.31, df=5, P=0.5051). Of the 25 individuals exposed to 1–5 ppm CFT Legumine, 21 (84%) survived to the 48-h experimental endpoint. There were no deaths during the first 8 h and there was zero mortality among five individuals exposed to the highest rotenone concentration. In contrast, two of the five larvae in the control were found dead, at the 36-h point and both were in the process of molting. The experiment ended at the 48-h endpoint, but all odonate survivors were still alive at 76 h.

Feeble shrimp

This native shrimp exhibited high tolerance to rotenone (Figure 3(D)). There was no significant difference in survival among the six different treatments (Log-Rank test, χ^2=4.24, df=5, P=0.5161). Of the 25 individuals exposed to 1–5 ppm CFT Legumine, 24 (96%) survived beyond the 48-h experimental endpoint. The one mortality among these five treatment groups was an individual that was observed to be molting during the 2 h period and subsequently found dead at the 4 h mark. There was zero mortality among

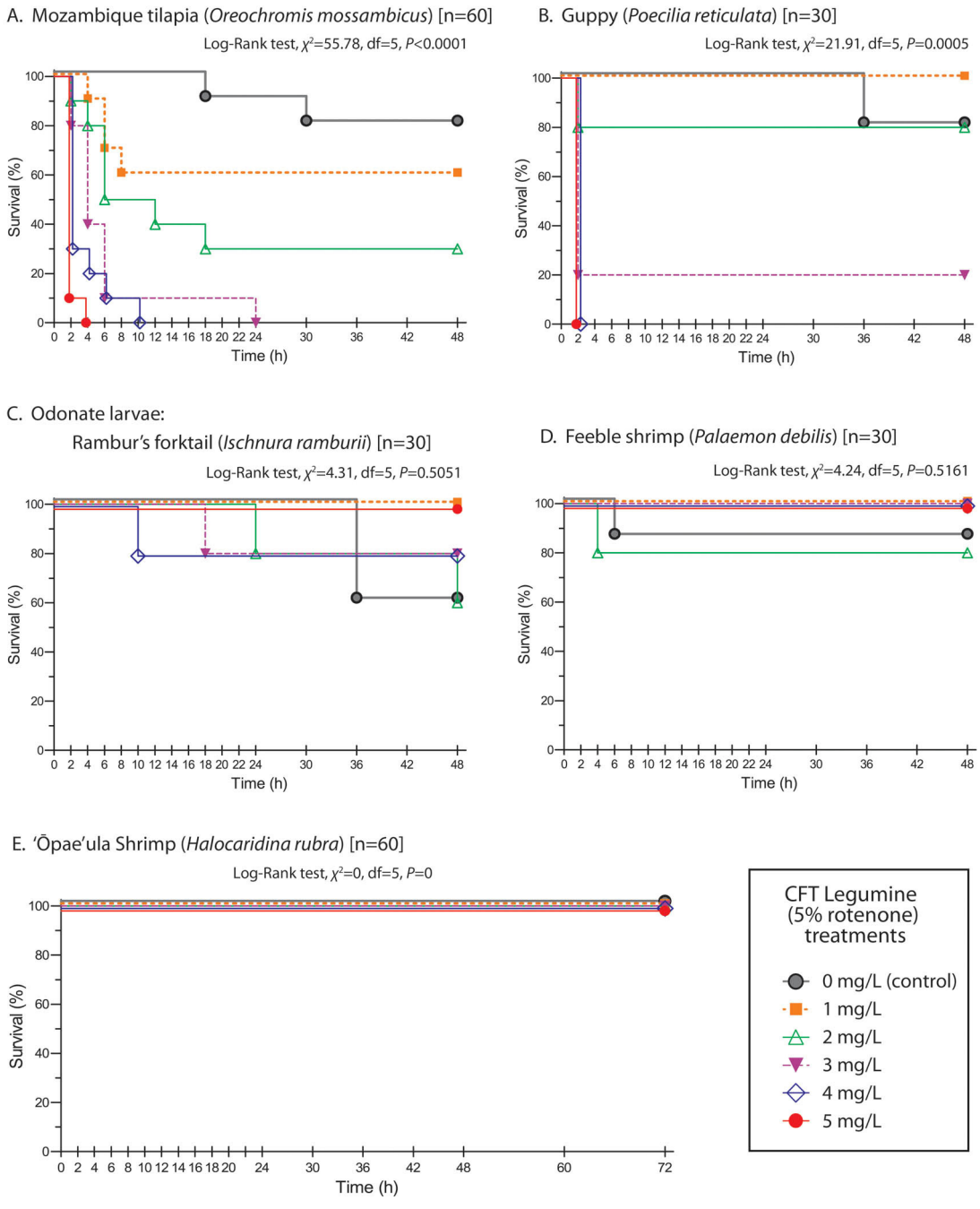

Figure 3. Kaplan-Meier survival analysis results for laboratory experiments comparing five species captured from 'Aimakapā Fishpond (or nearby anchialine pool) exposed to five different concentrations of CFT Legumine 5% rotenone and a control. The 5% rotenone concentrations shown in legend are equivalent to 0.05, 0.1, 0.15, 0.2 and 0.25 mg/L rotenone (i.e., active ingredient). All testing was conducted using brackish-water from site of capture. Each treatment included separate testing (1 individual/chamber) of 10 individuals of Mozambique tilapia and 'ōpae'ula shrimp and 5 individuals of each of the other three species. Horizontal axis displays elapsed time beginning with initial immersion (acute exposure) of test subjects with labeled tick marks indicating times when specimens were examined. The experiment was concluded at 48 h for all species, except 'ōpae'ula shrimp with endpoint of 72 h.

Table 2. Pairwise comparisons of Kaplan-Meier survivorship curves generated for Mozambique tilapia from ‘Aimakapā Fishpond wetland complex exposed for 48 h to five different concentrations of CFT Legumine™ (5% rotenone) and a control (see Figure 3(A)). The fifteen Mantel-Cox Log-Rank tests were calculated with WinSTAT® version 2012.1. Pairs of curves that were significantly different at Bonferroni corrected P<0.003 are in bold italics.

	CFT Legumine (5% rotenone) concentration				
	0 ppm (control)	1 ppm	2 ppm	3 ppm	4 ppm
Mozambique tilapia					
1 ppm	0.2457				
2 ppm	***<0.0001***	***<0.0001***			
3 ppm	***<0.0001***	***<0.0001***	0.0355		
4 ppm	***<0.0001***	***<0.0001***	***0.0021***	0.1455	
5 ppm	***<0.0001***	***<0.0001***	***<0.0001***	***0.0015***	0.1512

Table 3. Pairwise comparisons of Kaplan-Meier survivorship curves generated for five fish and invertebrate taxa exposed for 48 h to 4 and 5 ppm CFT Legumine™ (5% rotenone) (see Figure 4). The ten Mantel-Cox Log-Rank tests were calculated with WinSTAT® version 2012.1. Pairs of curves that were significantly different at Bonferroni corrected *P*<0.005 are in bold italics. All organisms tested were from ‘Aimakapā Fishpond wetland complex and vicinity.

Taxa	‘Ōpae’ula shrimp	Feeble shrimp	Odonate larvae	Guppy
‘Ōpae’ula shrimp				
Feeble shrimp	1.0			
Odonate larvae	0.1573	0.3173		
Guppy	***<0.0001***	***<0.0001***	***<0.0001***	
Mozambique tilapia	***<0.0001***	***<0.0001***	***<0.0001***	0.1353

the 15 individuals exposed to the three highest CFT Legumine concentrations (3–5 ppm) and in the 5 individuals in the 1 ppm concentration. In contrast, 4 of the 5 feeble shrimp in the control group survived to the 48-h experimental endpoint. The single control group mortality was an individual found on its side at the 4 h mark and confirmed dead at the 6 h mark, leading to suspicion that it had sustained an injury during capture or transfer. All 48-h survivors, except one, were still alive at 76 h.

Experiment session 2

‘Ōpae’ula shrimp

‘Ōpae’ula shrimp exhibited very high tolerance to rotenone. Over the 72-h exposure period, there was 100% survival in five rotenone treatment groups and the control group (Figure 3(E)). During the experimental period, there were no obvious behavioral differences among ‘ōpae’ula shrimp exposed to rotenone versus those in the control group. None of the treatment or control shrimp exhibited behaviors (e.g., laying on side, swimming continuously at surface) which might have suggested they were under stress. To ascertain if the 5 ppm CFT Legumine treatment water prepared for ‘ōpae’ula shrimp remained lethal to fish for an extended period, we subjected 10 guppies to the 72-h old, used test water and had 100% mortality in 2 hours.

Comparison of the five taxa

Mean and median survival times

Mozambique tilapia and guppies exposed to the higher concentrations of rotenone had the lowest mean survival estimates among the five taxa tested (Figure 4). Both fish species exhibited dose–response relationships for the five concentrations of CFT Legumine tested; in contrast, the invertebrates showed no such relationship. Odonate larvae and feeble shrimp had occasional mortalities in their respective control groups, but zero mortalities in the highest rotenone concentration. Log-Rank tests indicated the survival curves of control group versus the 5 ppm CFT Legumine treatment group for each of these two taxa were not significantly different (feeble shrimp: χ^2=1, df=1, P=0.3173; odonate larvae: χ^2=2.25, df=1, P=0.1336).

Survival in high rotenone concentrations

Laboratory tests indicate that 4 to 5 ppm CFT Legumine (i.e., 0.2 to 0.25 mg/L rotenone) would be sufficient to eradicate ‘Aimakapā’s tilapia population. To assess effects on targeted and non-targeted taxa, experimental data from the 4 and 5 ppm treatments were pooled. Comparison

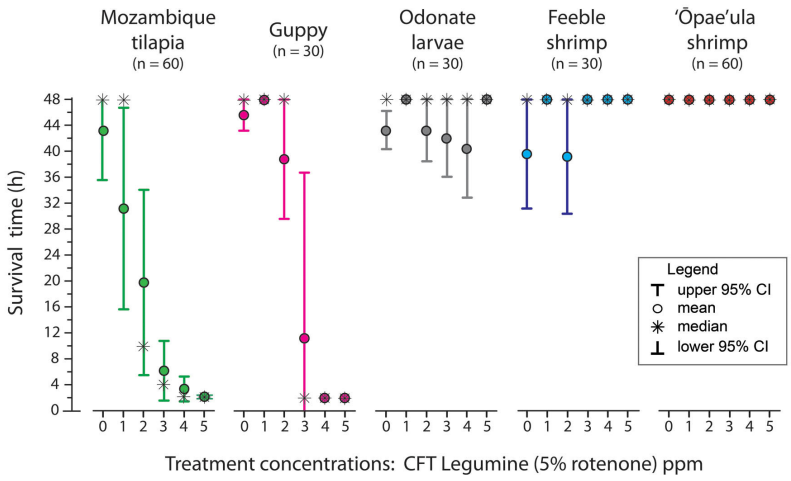

Figure 4. Comparison of mean and median survival times (hours) of five species exposed to five different concentrations of CFT Legumine 5% rotenone and a control. All testing was conducted using brackish-water from sites of capture, 'Aimakapā Fishpond for the first four species and an anchialine pool for 'ōpae'ula shrimp. For this comparison, experiment endpoint was 48 h for all species, although 'ōpae'ula shrimp were observed for a total of 72 h (with all surviving).

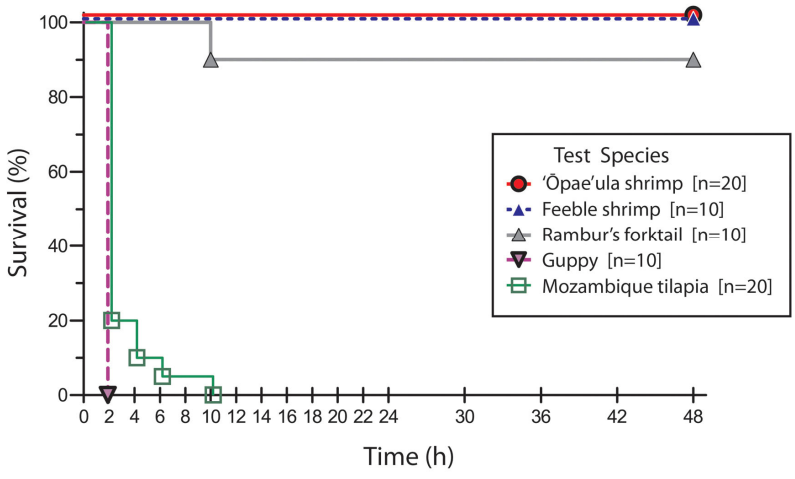

Figure 5. Kaplan-Meier survival analysis showing response of five species to the higher concentrations of CFT Legumine 5% rotenone. For each species, experimental data from the 4 and 5 ppm CFT Legumine treatments were pooled. All testing was conducted using brackish-water from sites of capture. Labeled tick marks on the x-axis indicate the times when specimens were examined. For this comparison, experiment endpoint was 48 h for all species.

of resulting survival curves (Figure 5) revealed significant variation among the five different taxa (Log-Rank test, χ^2=80.36, df=4, P<0.0001). Pairwise comparisons of the survivorship curves demonstrated differences in response (Table 3). Log-Rank tests comparing the survivorship curves of the different species pairs showed that survival by each of the two fish species was significantly different from each of the three invertebrate taxa. In contrast, the survival curves of Mozambique tilapia and guppies, both highly sensitive to rotenone, were not significantly different (Log-Rank test, 4–5 ppm pooled data: χ^2=2.23, df=1, P=0.1353). Similarly, Log-Rank tests paired comparisons of the pooled 4–5 ppm treatment survival curves of the three invertebrate species, all highly tolerant to rotenone, were not significantly different (odonate larvae versus feeble shrimp: χ^2=1, df=1, P=0.3173; odonate larvae versus 'ōpae'ula shrimp: χ^2=2, df=1, P=0.1573; and feeble shrimp versus 'ōpae'ula shrimp: χ^2=0, df=1, P=1.0).

Loading

Although individual loading ratios were generally high in rotenone tests conducted with tilapia, a multiple regression analysis indicated that the influence of loading was not significant relative to differences in tilapia survival times (Table 1, Figure 6). In a multiple regression analysis, rotenone concentration and loading explained 57% of the variance in survival time (R^2 = 0.57; adjusted R^2 = 0.56, F(2, 60) = 38.23, df = 57, p <0.0001). Rotenone concentration was inversely related to survival time and highly significant

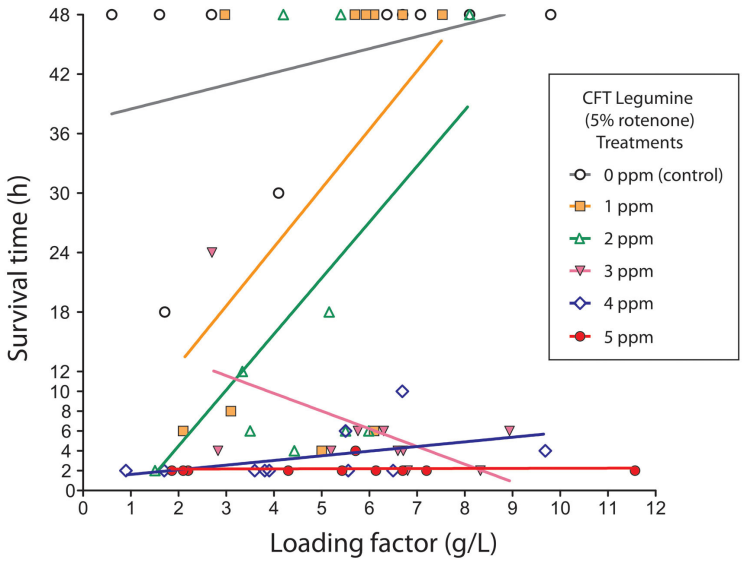

Figure 6. Relationship between survival time and loading (i.e., ratio of organism mass to test water volume) for rotenone exposure experiments conducted with Mozambique tilapia. A simple linear regression line was plotted and is displayed for each of the six treatment groups, exposed to five different concentrations of CFT Legumine 5% rotenone and a control. Each treatment group consisted of a mix of 10 juvenile and adult tilapia individually tested. The experiment endpoint was 48 h. Also refer to Table 1.

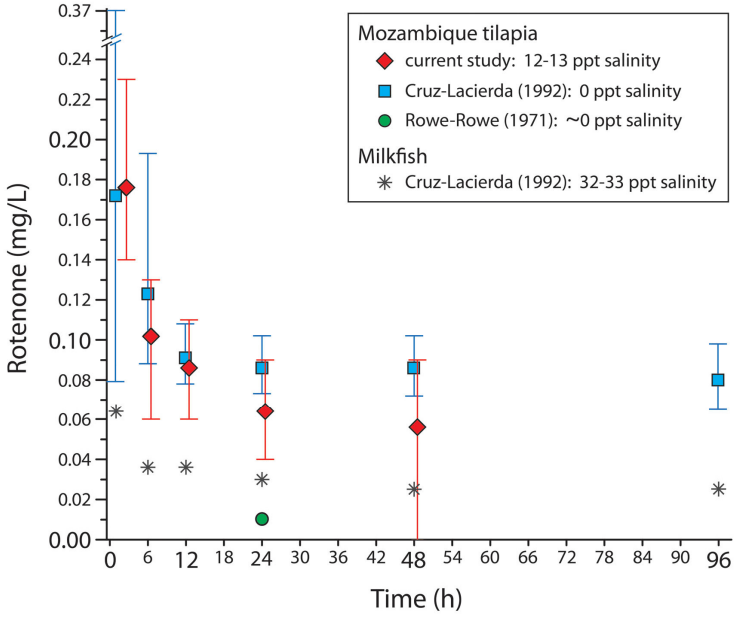

Figure 7. Rotenone concentrations lethal to 50% (LC_{50}) of Mozambique tilapia at different time periods based on toxicity tests of current study conducted in brackish water (mean 24 °C) compared to published results for small Mozambique tilapia tested in fresh water and small juvenile milkfish in salt water (Rowe-Rowe 1971; Cruz-Lacierda 1992). All LC_{50} values were calculated by probit analysis and based on rotenone active ingredient concentrations. Corresponding 95% confidence intervals (CIs) for tilapia are displayed (CIs not provided by Rowe-Rowe 1971). Water temperatures among studies differed: 21–26 °C (mean 24 °C) for current study; 22 °C for tilapia of Rowe-Rowe (1971) study; 28-29 °C for tilapia and 27–28 °C for milkfish tests of Cruz-Lacierda (1992) study.

(β = -8.705, p <0.0001), whereas loading was not significant (β = 1.066, p = 0.1473). The two predictor variables, loading and rotenone concentration, were found to be independent of each other (R^2 = <0.75), indicating multicollinearity was not a problem. Rotenone concentration in a simple linear regression explained 56% of the variation in tilapia survival (R^2 = 0.56; adjusted R^2 = 0.55, F(1, 60) = 72.86, *df* = 58, p <0.0001).

Median lethal concentration (LC_{50})

Mozambique tilapia was the only taxa tested where data were appropriate for LC_{50} calculations. Based on probit analysis, the 2, 6, 12, 24, and 48 h LC_{50} values for Mozambique tilapia were 0.18, 0.10, 0.09, 0.06, and 0.06 mg/L rotenone (i.e., 3.53, 2.05, 1.73, 1.27, and 1.12 ppm CFT Legumine) (Figure 7). All regressions exceeded

goodness of fits R-square >0.90. The derived LC_{50} values, along with 95% CIs, are graphically compared with previously published LC_{50} values from laboratory rotenone toxicity experiments with Mozambique tilapia tested in fresh water and for juvenile milkfish tested in salt water (Figure 7). The calculated 2, 6, 12, and 24 h LC_{50} for guppies each produced a value of 0.12 mg/L rotenone, and the LC_{50} for 48 h was 0.11 mg/L. However, the 95% confidence limits extended to infinity due to small sample size and death of a control.

Discussion

Mozambique tilapia

The current laboratory study demonstrated that CFT Legumine (5% rotenone) concentrations >3 ppm (i.e., > 0.15 mg/L rotenone) were effective in killing juvenile and adult Mozambique Tilapia in brackish water, but had little or no effect on the survival of invertebrates tested. However, results suggested that Mozambique tilapia are more tolerant than most other fish species based on our review of literature (see Meadows 1973; Finlayson et al. 2010b). Few published laboratory and field studies exist on the effects of rotenone on Mozambique tilapia or on tilapia in general and the few that are published provide little useful information. Two previous laboratory studies, both static tests in freshwater conditions, were conducted with small or juvenile Mozambique tilapia and results given in terms of LC_{50} values (Rowe-Rowe 1971; Cruz-Lacierda 1992). The LC_{50} values calculated by Cruz-Lacierda (1992) were consistent with those of the current study, and showed that Mozambique tilapia has relatively high tolerance compared with other fishes tested. In contrast, Rowe-Rowe (1971) reported a 24-h LC_{50} value of 0.0103 mg/L rotenone for Mozambique tilapia, indicative of moderate rotenone tolerance, 6 to 8 times less than that indicated by the 24-h LC_{50} values of the current study (0.064 mg/L) and that of Cruz-Lacierda (0.086 mg/L). By comparison, the 24-h LC_{50} values reported in the literature for six different freshwater fish species range from a low of 0.002 mg/L for rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792) to a high of 0.02 mg/L for channel catfish *Ictalurus punctatus* (Rafinesque, 1818) (Finlayson et al. 2010b).

Many early published studies on rotenone tolerance of aquatic organisms are short on details about methods and other critical information. Results from separate studies can be difficult or impossible to compare because of differences in time periods used in calculating LC_{50} values (e.g., 24-h versus 96-h). The toxicity of different rotenone formulations are also known to vary (Finlayson et al. 2010b; 2012). In his work with Mozambique tilapia, Rowe-Rowe (1971) did not provide confidence intervals for the 24-h LC_{50} value obtained and no other LC_{50} time period was calculated. The low 24-h LC_{50} value from that study may have resulted from any number of factors, such as poor experimental design, low dissolved oxygen, buildup of metabolic wastes, condition of fish, or perhaps characteristics of the rotenone used, a *Derris* powder consisting of 6.5% rotenone. Cruz-Lacierda (1992) derived tested dilutions from analytical grade rotenone (90–95% pure). Unfortunately, neither Rowe-Rowe nor Cruz-Lacierda provides enough details to allow full evaluation of their methods and experimental designs. In particular, the rotenone concentrations actually tested by Rowe-Rowe (1971) is unclear. It is noteworthy that LC_{50} values determined and reported by Rowe-Rowe (1971) for other fish species also appear relatively low compared to values reported by other researchers testing those same species.

Tilapia, as a group, are generally considered more tolerant to rotenone than many other fish species (Metzger and Shafland 1986); but a literature review suggests such conclusions are based heavily on anecdotal observations. Few controlled laboratory exposure studies have been conducted and the precise rotenone formulations and treatment concentrations used in the field to kill tilapia are rarely reported even though the chemical has frequently been used to control tilapia, including Mozambique tilapia (St. Amant 1966; Nico and Walsh 2011; Tourenq et al. 2011; Russell et al. 2012). In addition, most tilapia eradication projects have targeted populations inhabiting freshwater lentic habitats. Two recent eradication projects targeting Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758) are worth mentioning because information is known about the rotenone formulation and the actual or likely treatment concentrations used. One of the projects, designed to achieve a treatment level of 0.25 mg/L rotenone, resulted in the successful eradication of Nile tilapia from a natural crater lake in the Galapagos (Nico and Walsh 2011; LGN, unpublished data). The other project removed most of the Nile tilapia inhabiting a system of small ponds and ditches in an aquaculture facility in coastal Mississippi, USA (Schofield et

al. 2007). The project in Mississippi involved application of Prentox© rotenone solution at a product treatment concentration of 5 ppm. Although not stated, it is reasonable to assume that the formulation consisted of 5% rotenone which would equate to a rotenone field treatment concentration of 0.25 mg/L.

Guppies

Guppies exhibited moderate tolerance to rotenone relative to other fish species. Resulting survival analysis graphs generally indicated that guppies were somewhat less tolerant to the chemical than Mozambique tilapia (Figures 3 and 5), although statistical analysis revealed no significant differences in the responses by the two species to the higher rotenone concentrations tested (Table 3). An in-depth literature search revealed no previous studies on the effects of commercial-grade rotenone on guppies, although there have been a few laboratory studies that tested powder or extracts that the researchers themselves obtained from rotenoid-bearing plants. Unfortunately, information provided is incomplete, lacking critical details about methods, therefore requiring caution when interpreting results and conclusions. The earliest documented studies are those of Pagán (1948) and Barnes and Freyre (1966), both exposed adult male guppies to extracts of rotenoid-bearing plants under freshwater, or presumably freshwater conditions. Pagán (1948) examined the toxicity of the roots of *Lonchocarpus nicou* (Aubl.) DC. and *Derris elliptica* Benth. (now considered a junior synonym of *Paraderris elliptica* (Wall.) Adema). Although the LC_{50} value was not calculated, Pagán provided sufficient data to allow readers to estimate a 6-h LC_{50} of about 0.066 mg/L rotenone. That value suggests moderate sensitivity to the chemical by guppies relative to many other fishes. In contrast, we calculated a 6-h LC_{50} of 0.12 mg/L rotenone for guppies from ʻAimakapā, evidence of much greater tolerance. The marked difference between our 6-h LC_{50} and the LC_{50} value estimated from Pagán's data may be due to numerous factors, although there was no statistical difference given the broad confidence limits associated with our calculated LC_{50} value.

Barnes and Freyre (1966) reportedly followed the methods of Pagán in their test of leaf extracts of the rotenone-containing plant *Tephrosia vogelii* Hook. f. They documented mortality, but found that lethality differed depending on extraction method used. In addition, variation was found in the lethality of individual plants. Unfortunately, LC_{50} was not given and since test time duration and other details were not reported, it is not possible for others to estimate an LC_{50} value. A later study, by Guerrero et al. (1990) consisted of laboratory bioassays to test the effectiveness of *Derris elliptica* root powder for possible eradication of adult guppies at an aquaculture facility in the Philippines. Results showed that 5 mg/L of the root powder was sufficient in fresh water, but higher concentrations were needed at higher salinities, 10–20 mg/L at 10 ppt salinity and 30 mg/L at 20 ppt. The rotenone concentration in the root powder used by Guerrero et al. (1990) was not reported, so it is not reasonable to compare their results with other studies.

Invertebrates

In marked contrast to fish species tested, little or no mortality was observed among the three invertebrate taxa exposed to 1 to 5 ppm CFT Legumine (i.e., 0.05 to 0.25 mg/L rotenone): 16% mortality for odonate larvae, 4% for feeble shrimp, and 0% for ʻōpaeʼula shrimp. For each of these three invertebrates, survival in the highest rotenone exposures was not statistically significantly different from the control. Because of the high survival of invertebrates during exposure tests, it was not possible to calculate LC_{50} values for any of the invertebrates.

<u>Odonate larvae</u>

Larvae and adults of the non-native *Ischnura ramburii* were the most abundant odonates in the ʻAimakapā wetland complex and rotenone exposure tests demonstrated that larvae of the species were highly tolerant. Field surveys conducted in association with laboratory experiments indicated at least six odonate species, 2 indigenous and 4 non-natives, inhabit ʻAimakapā or nearby areas (RAE, unpublished data). Although none of the native odonates found in ʻAimakapā is federally listed as threatened or endangered, an endemic Hawaiian damselfly, *Megalagrion xanthomelas*, has been recorded from anchialine pools in KAHO and is a candidate for listing by the U.S. Fish and Wildlife Service.

Most published field and laboratory studies indicate that larvae of freshwater odonates tend to be highly tolerant of rotenone at concentrations normally used to kill fish (Leonard 1939; Brown and Ball 1943; Claffey and Ruck 1967; Watkins and Tarter 1974; Engstrom-Heg et al. 1978; Serns 1979; Demong 2001). For example, experimental

treatment of Third Sister Lake, Michigan (USA), with powdered *Derris* root revealed that larvae of Zygoptera and most Anisoptera were relatively resistant (Brown and Ball 1943). In a laboratory study with Noxfish® (5% rotenone), odonate larvae of *Macromia* were moderately to highly tolerant, with 24-h and 96-h LC_{50} values of 4.7 and 1.0 ppm Noxfish®, respectively, equivalent to 0.24 and 0.05 mg/L rotenone (Chandler and Marking 1982; see Finlayson et al. 2010b). In contrast, laboratory tests with the odonate *Basiaeschna janata* (Say, 1839) resulted in a 96-h LC_{50} of 0.22 mg/L rotenone, leading researchers to conclude that the species may be harmed during rotenone treatments targeting fish (Watkins and Tarter 1974). Minor sampling of South Africa's Rondegat River during pre- and post-rotenone treatment suggested rotenone may have caused a decline in odonates (Woodford et al. 2013).

Feeble shrimp

All feeble shrimp tested during the current study were from 'Aimakapā, the only shrimp known to inhabit the wetland complex. Our review of the literature uncovered no published information on possible effects of rotenone on this species. However, earlier laboratory and field studies do provide evidence that freshwater and marine palaemonid shrimp, and many other macrocrustaceans, have a much greater tolerance of rotenone than fish (Combette and Legendre 1937; Gilmore et al. 1981; Næss et al. 1991; Cruz-Lacierda 1993; Ogunsanya et al. 2011).

'Ōpae'ula shrimp

'Ōpae'ula shrimp do not inhabit 'Aimakapā, but are common in nearby small anchialine pools. Based on a literature review, there have been no previous laboratory experiments evaluating the lethality of rotenone on this species. However, there has been a number of small-scale rotenone projects conducted in the Hawaiian Islands aimed at removal of invasive fish from small anchialine pool habitats and observers report that 'ōpae'ula shrimp and other native invertebrate populations seem to rapidly recover after the chemical treatment (Brock and Kam 1997; Yamamoto and Tagawa 2000; Chai and Mokiao-Lee 2008). To explain such observations, participants concluded that the surviving 'ōpae'ula shrimp had avoided rotenone's harmful effects by taking temporary refuge in subterranean cavities, returning to surface waters only after the rotenone had degraded to non-lethal concentrations (Brock and Kam 1997; Yamamoto and Tagawa 2000; Chai and Mokiao-Lee 2008). Results from experiments of the current study suggest that the survival of all or most of the 'ōpae'ula shrimp in those earlier treated pools was due to the shrimp's high tolerance to the chemical rather than by avoidance of the chemical.

Meager information exists concerning rotenone and its effects on other atyid shrimps. For instance, comparison of drift of macroinvertebrates from treated and untreated reaches of two Papua New Guinea streams indicated that rotenone induced some atyid drift (Dudgeon 1990), but the significance is unclear. A laboratory study designed to assess the effects of rotenone pellets on target fish and non-target organisms showed that atyid shrimp were not harmed even though they appeared to be feeding on the pellets (Gehrke 2003).

Rotenone lethality in brackish and marine waters

Experimental results of the current study, together with experimental results of Cruz-Lacierda (1992), who conducted tests in freshwater conditions, suggest the exposure response of Mozambique tilapia is similar regardless of salinity (Figure 7). In contrast, Wilson (1990) who was interested in controlling fish predators in fresh- and brackish-water impoundments used for aquaculture, stated that higher dosages of rotenone are required for high salinity waters. However, Wilson did not specifically mention tilapia or any other fish species and his conclusion appears to be largely based on a few anecdotal observations. The only previous study we found is Guerrero et al. (1990) who explored use of indigenous ichthyotoxic plants for management of fresh- and brackish-water aquaculture ponds in the Philippines. For their laboratory experiments, the researchers selected guppies as a test animal and used *Derris* powder processed from the dried roots of *D. elliptica* plants. Experimental treatments included four different *Derris* powder concentrations (i.e., 5, 10, 20, and 30 mg/L) and three different salinities ranging from fresh to 20 ppt, with tests run at water temperatures of 25 to 26 °C. Bioassay results indicated that, as salinity increased, higher *Derris* powder concentrations were needed to kill guppies within a 2 h period: 5 mg/L of the powder in fresh water; 10–20 mg/L at 10 ppt salinity); and 30 mg/L at 20 ppt. Unfortunately, Guerrero et al. did not provide the actual rotenone concentration of the root powder used in the study, likely because it was not determined. Many

other details on the methodology are absent in the paper so a complete assessment of the experiment is not possible.

In general, the few laboratory and field studies that have evaluated effects of rotenone on marine and estuarine fish indicate low tolerance, with death occurring at concentrations that are within the range lethal to many freshwater fish species (Combette and Legendre 1937; Gilmore et al. 1981; Hegen 1985; Cruz-Lacierda 1992), although some marine fish may be especially sensitive (Næss et al. 1991; Wingard and Swanson 1992). However, considering differences in study design and taxa tested, the accumulated data suggest the levels of rotenone lethal to fish in brackish and marine waters are not much different than that of fresh water. In his review of the use of rotenone in fisheries research, Krumholz (1948) stated that powdered *Derris* root was quite effective in collecting small fishes in salt and brackish waters, but gave no dosage levels. Based on laboratory and field work with estuarine fishes from the Gulf of Mexico, Hegen (1985) concluded that a concentration of 0.4 mg/L rotenone in the form of Noxfish® (5% rotenone) was the minimum concentration necessary to cause 100% mortality of fishes in brackish water of 15–20 ppt salinity and 24.5–30.1 °C. A concentration of 0.4 mg/L is moderately high, but is well within the range (0.1–3.0 mg/L) regularly used to kill fish in freshwater systems.

Ichthyotoxic plants

Ichthyotoxic plants, including *Tephrosia*, that contain high concentrations of rotenone, have long been used by Hawaiians and other Pacific islanders as a traditional method for capture of food fish (Stokes 1921; Merrill 1943; Cox 1979; Armstrong et al. 2011). Using plants to eradicate tilapia from ʻAimakapā may be more culturally acceptable to Hawaiians than application of commercial rotenone formulations, but the quantity of *Tephrosia* required to treat a site as large as ʻAimakapā is not commercially available. Moreover, the toxic content in piscicidal plants can vary widely, depending on such factors as locality and season (Quigley 1956), and among individual plants of the same species (Barnes and Freyre 1966). For instance, Jones (1933) reported that the rotenone content of *Derris elliptica* can vary from 0 to about 7 percent and that of plants of the genus *Lonchocarpus* from <1 to about 11 percent.

For modern fish management, rotenone is obtained from commercial sources, manufacturers who extract rotenone from the roots, seeds and leaves of selected plants and then produce formulations of known concentrations, either as crystalline preparations, emulsified solutions, or dusts (Finlayson et al. 2010a). For natural resource managers and fisheries biologists interested in eradicating invasive fishes, use of such commercial products allows much greater control in achieving a particular rotenone concentration. In contrast, traditional methods typically involve a complex and time-consuming process of finding, collecting, and transporting sufficient numbers of rotenone-bearing plants to the work site and then cutting, pounding, and crushing the plants on the shore to release the poison into the water (Stokes 1921; Barrau 1955; Cox 1979). Because rotenone concentration is unknown in traditional methods, there is greater risk of unfavorable outcomes, either too small a dose whereby targeted fish survive, or "overdosing" of the treated environment with a chance of unintended consequences.

Current regulations

The situation involving possible use of rotenone in ʻAimakapā Fishpond and any of the many small anchialine pools of the Hawaiian Islands invaded by non-native fishes is problematic because most of these sites are brackish water. In 2007, the US Environmental Protection Agency (USEPA) completed their review of rotenone as part of their reregistration eligibility determination (RED) for the chemical (USEPA 2007). The EPA's final decision placed wider restrictions on use of the chemical in the USA, stipulating that rotenone's only approved use is for fish management operations (i.e., fish control and sampling) conducted in fresh water (i.e., lakes, ponds, reservoirs, and streams).

The new regulations specifically prohibit use of rotenone in marine and estuarine environments. According to those involved in the review process, the paucity of published experi-mental data on the toxic effects of rotenone on brackish-water and marine organisms was a main factor in the new restrictions. However, depending on the habitat and whether adequate site-specific toxicity data are available, it still may be possible to apply for and receive approval for a variance, called a Special Local Need (SLN) 24(c) registration (B. Finlayson, personal communication).

Proposed field treatment concentration

The lowest laboratory concentration resulting in 100% mortality of tilapia within 24 h was 0.15 mg/L rotenone or 3 ppm CFT Legumine (Figure 3). However, a higher concentration is typically necessary to achieve eradication in the field because environmental factors can affect the chemical. The American Fisheries Society Standard Operating Procedures Manual recommends treating at a concentration at least twice the Minimum Effective Dose (MED) based on 8 hours exposure (Finlayson et al. 2010a). In our experiments, all Mozambique tilapia were dead by 4 hours in the 0.24 mg/L concentration and by 10 hours in 0.2 mg/L rotenone, which suggests an 8 hour MED exposure of about 0.22 mg/L. Doubling that value is 0.44 mg/L rotenone or 8.8 ppm CFT Legumine which is excessive.

An anonymous reviewer suggested a minimum field treatment concentration could be calculated by first doubling our estimated 48-h LC_{50} of 0.06 mg/L to obtain an approximate 48-h LC_{100} value and then doubling the resulting LC_{100} value. That methodology suggests a minimum treatment of 0.24 mg/L rotenone or 4.8 ppm CFT Legumine to eradicate ‘Aimakapā’s tilapia population. Such a concentration seems reasonable since it is near the 0.25 mg/L rotenone concentration that we used during a recent project in the Galapagos to successfully eradicate Nile tilapia populating a crater lake (LGN, unpubl. data). We estimate ‘Aimakapā’s total water volume to be approximately 31,100 m^3, which would require 149.3 L CFT Legumine (5% rotenone) to achieve a 0.24 mg/L rotenone concentration or 4.8 ppm CFT Legumine.

Implications for ‘Aimakapā

A rotenone concentration necessary to eradicate Mozambique tilapia inhabiting ‘Aimakapā would likely kill other fishes present. However, the three native fish species populating the water body are widespread and common in Hawaii and could be removed and later restocked. Our tests demonstrated that shrimp and odonate populations would be little affected. Other aquatic invertebrates were not tested, although the literature indicates most invertebrates are more tolerant of rotenone than fish (Finlayson et al. 2000; Ling 2003; Vinson et al. 2010). Degree of sensitivity depends on: the particular taxa and life stage; physical, chemical, and biological characteristics of the waterbody; and concentration of rotenone applied (Schnick 1974; Marking and Bills 1976; Chandler and Marking 1982; Næss 1991; Finlayson et al. 2000). In addition, because ‘Aimakapā has degraded over the years due to a combination of increased sedimentation, worsening water quality, and invasion by non-native species, its current aquatic invertebrate fauna consists of common, widespread taxa tolerant of poor conditions. Consequently, any decline in numbers caused by rotenone treatment would likely be short term and invertebrate diversity may increase following tilapia removal.

The ‘Aimakapā environment includes certain characteristics that increase the likelihood that use of rotenone would result in successful eradication of tilapia, but the site also presents certain challenges. ‘Aimakapā is amenable to rotenone treatment because the wetland is moderately small, shallow, and a closed surface-water system. Complicating factors that need to be considered include: 1) the extensive amount of emergent vegetation and detrital mats which may hinder rotenone application and dispersion and also afford refugia for the tilapia; 2) the high amounts of accumulated detritus and highly suspendable sediments, all of which may adsorb some rotenone thereby reducing the amount of rotenone in the water column; 3) the site’s complex groundwater hydrology created by the highly permeable volcanic rock substrate that allows passage of a mix of fresh and salt water and results in tide-related fluctuations of surface water levels; and 4) the presence of federally-protected waterbirds during all or most of the year.

The marsh vegetation at ‘Aimakapā is mostly invasive plants that the National Park Service intends to remove. If rotenone is used to eradicate the tilapia, its effectiveness would be increased if application were delayed until after most of the invasive plants are removed. The potential movement of rotenone through fractured and permeable volcanic rock will likely become an issue if rotenone is chosen for tilapia removal, especially given ‘Aimakapā’s proximity to the ocean and nearby shallow reefs. As yet, no dye studies have been conducted in ‘Aimakapā to characterize groundwater hydrology.

Implications for anchialine pools

There have been successful removals of invasive fishes from Hawaiian anchialine pools in the past (Brock and Kam 1997; Yamamoto and Tagawa 2000; Chai and Mokiao-Lee 2008; Carey et al. 2011). However, all or most were conducted with little or no pre-treatment experiments to determine what minimum concentrations of rotenone

were necessary for removal of targeted fish and to what extent native aquatic organisms might be harmed. ‘Ōpae’ula shrimp are considered a keystone herbivorous species in anchialine pool systems (Bailey-Brock and Brock 1993; Dalton et al. 2013). Consequently, results from the current experiments documenting that this endemic shrimp is highly tolerant of rotenone is important for resource managers deciding whether to use the chemical to remove invasive fish from anchialine pools.

Although traditional “fish poisoning” using ichthyotoxic plants in a water body the size of ‘Aimakapā to remove tilapia may not be technically feasible, such traditional methods would be possible and perhaps more culturally acceptable for removal of invasive fishes from small anchialine pools.

Conclusions

Prior to our experiments, it was generally assumed that concentrations of rotenone necessary to kill invasive fish in Hawaiian brackish-water wetlands would also be harmful to endemic macro-invertebrates, including ‘ōpae’ula shrimp, a keystone anchialine species. Our experiments showed that ‘ōpae’ula shrimp and other macro-invertebrates tested were essentially unaffected by the chemical at levels that were lethal to non-native fishes. Thus rotenone could control tilapia and other invasive fishes without disrupting key invertebrates in these systems.

Although rotenone’s effects on freshwater organisms have been well studied, the current research represents one of only a few controlled laboratory experiments quantitatively assessing rotenone tolerance of brackish or marine fauna. Rotenone applied to ‘Aimakapā would kill all or most of the few native fishes present. However, it is conceivable that the few dozen milkfish and bluefin trevally inhabiting ‘Aimakapā could be captured prior to rotenone application and then later released back into the site after the tilapia are removed and the rotenone has degraded. Although laboratory tests indicated shrimp and odonates would likely not be harmed, rotenone use may cause the temporary decline of some of the aquatic invertebrates taxa present in the site but not tested. However, it is also recognized that Mozambique tilapia and guppies, two highly invasive fish species, are abundant and widespread in ‘Aimakapā and predation by these two species is likely already having a serious negative impact on the invertebrate fauna.

The longer Mozambique tilapia persist in ‘Aimakapā, the harm they may be causing to the site’s invertebrate fauna will continue or perhaps worsen. If Mozambique tilapia in ‘Aimakapā invade other sites in KAHO, then the invertebrates of invaded sites will be exposed to additional predation pressure. Of particular concern is the likelihood that the tilapia will gain access to nearby anchialine pools, perhaps during future inevitable flood events. Woodford et al. (2013), recognizing the serious threat posed by invasive fish to native fish and aquatic insect communities, concluded that the long-term positive conservation impact of removing invasive fish species outweighs the short-term negative effects of piscicides such as rotenone. Our findings have important implications for use of rotenone for the management of invasive fishes in ‘Aimakapā, anchialine pools throughout Hawai’i, and brackish/marine habitats in other parts of the world.

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References

Armstrong K, Herdrich D, Levine A (2011) Historic fishing methods in American Samoa. U.S. Department of Commerce, National Oceanic and Atmospheric Administration (NOAA) Technical Memorandum, NMFS-PIFSAC-24, Honolulu, Hawaii, 70 pp

ASTM (American Society for Testing and Materials) (2007) Standard Guide for Conducting Acute Toxicity Tests on Test

Materials with Fishes, Macroinvertebrates, and Amphibians. ASTM Standard E729-96 (reapproved 2007). American Society for Testing and Materials International, West Conshohocken, Pennsylvania

Bailey-Brock JH, Brock RE (1993) Feeding, reproduction, and sense organs of the Hawaiian anchialine shrimp *Halocaridina rubra* (Atyidae). *Pacific Science* 47: 338–355

Barnes DK, Freyre RH (1966) Recovery of natural insecticides from *Tephrosia vogelii*. II. Toxicological properties of rotenoids extracted from fresh and oven-dried leaves. *Economic Botany* 20: 368–371, http://dx.doi.org/10.1007/BF02904057

Barrau J (1955) Fishing poisons of the South Pacific. *South Pacific Commission Quarterly Bulletin* 5: 7–8

Bienfang P, DeFelice S, Laws EA (2011) Quantitative environmental benchmarking in a hydrologically driven Hawaiian coastal system. *Marine Technology Society Journal* 45: 88–100, http://dx.doi.org/10.4031/MTSJ.45.2.10

Bland JM, Altman DG (2004) The logrank test. *British Medical Journal* 328: 1073, http://dx.doi.org/10.1136/bmj.328.7447.1073

Brock RE, Kam AKH (1997) Biological and water quality characteristics of anchialine resources in Kaloko-Honokohau National Historical Park. Technical Report 112. Cooperative National Park Resources Studies Unit, University of Hawaii, Honolulu, Hawaii, 115 pp

Brown CJD, Ball RC (1943) An experiment in the use of *Derris* root (rotenone) on the fish and fish-food organisms of Third Sister Lake. *Transactions of the American Fisheries Society* 72: 267–284, http://dx.doi.org/10.1577/1548-8659(1942)72[267:AEITUO]2.0.CO;2

Capps KA, Turner CB, Booth MT, Lombardozzi DL, McArt SH, Chai D, Jr. NGH (2009) Behavioral responses of the endemic shrimp *Halocaridina rubra* (Malacostraca: Atyidae) to an introduced fish, *Gambusia affinis* (Actinopterygii: Poeciliidae) and implications for the trophic structure of Hawaiian anchialine ponds. *Pacific Science* 63: 27–37, http://dx.doi.org/10.2984/1534-6188(2009)63[27:BROTES]2.0.CO;2

Carey CC, Ching MP, Collins SM, Early AM, Fetzer WW, Chai D, Hairston NG, Jr (2011) Predator-dependent diel migration by *Halocaridina rubra* shrimp (Malacostraca: Atyidae) in Hawaiian anchialine pools. *Aquatic Ecology* 45: 35–41, http://dx.doi.org/10.1007/s10452-010-9321-0

Carlton JT, Eldredge LG (2009) Marine bioinvasions of Hawai'i. *Bishop Museum Bulletin in Cultural and Environmental Studies* 4: 1–203

Chai D, Mokiao-Lee A (2008) Reviving a native anchialine community: A case study of rotenone use to eradicate *Gambusia affinis* in two anchialine pools at Hualalai Resort, Kaupulehu-Kona, Hawaii (abstract). In: Proceedings of the 89th Annual Meeting of the American Association for the Advancement of Science Pacific Division, Program with Abstracts, Volume 27, Part I. Hawaii Preparatory Academy on the Big Island, Waimea, Hawaii, June 15–20, 2008. American Association for the Advancement of Science, p 63

Chandler JH, Jr., Marking LL (1982) Toxicity of rotenone to selected aquatic invertebrates and frog larvae. *Progressive Fish Culturist* 44: 78–80, http://dx.doi.org/10.1577/1548-8659(1982)44[78:TORTSA]2.0.CO;2

Claffey FJ, Ruck JE (1967) The effect of rotenone on certain fish food organisms. *Proceedings of the Southeastern Association of Fish and Wildlife Agencies* 20 (1966): 278–283

Combette R, Legendre R (1937) Actions de la roténone sur quelques animaux marins. *Comptes Rendus Hebdomadaires des Séances et Mémoires de la Société de Biologie et des ses Filiales (Paris)* 124: 49–50

Cox PA (1979) Use of indigenous plants as fish poisons in Samoa. *Economic Botany* 33: 397–399, http://dx.doi.org/10.1007/BF02858335

Cruz-Lacierda ER (1992) Toxicity of rotenone to milkfish, *Chanos chanos*, and tilapia, *Oreochromis mossambicus*. In: Shariff M, Subasinghe RP, Arthur JR (eds), Diseases in Asian Aquaculture I, Proceedings of the 1st symposium on diseases in Asian aquaculture, Bali, Indonesia, November 26–29, 1990. Fish Health Section, Asian Fisheries Society, Manila, Philippines, pp 419–423

Cruz-Lacierda ER (1993) Effect of rotenone and saponin on the shell quality of juvenile tiger shrimp, *Penaeus monodon*. *Israeli Journal of Aquaculture Bamidgeh* 45: 126–130

Dalton CM, Mokiao-Lee A, Sakihara TS, Weber MG, Roco CA, Han Z, Dudley B, MacKenzie RA, Hairston NG (2013) Density- and trait-mediated top–down effects modify bottom–up control of a highly endemic tropical aquatic food web. *Oikos* 122: 790–800, http://dx.doi.org/10.1111/j.16000706.2012.20696.x

Demong L (2001) The use of rotenone to restore brook trout in the Adirondack Mountains of New York—an overview. In: Cailteux RL, DeMong L, Finlayson BJ, Horton W, McClay W, Schnick RA, Thompson C (eds), Rotenone in Fisheries: Are the Rewards Worth the Risks? American Fisheries Society, Bethesda, Maryland, pp 29–35

Dudgeon D (1990) Benthic community structure and the effect of rotenone piscicide on invertebrate drift and standing stocks in two Papua New Guinea streams. *Archiv fuer Hydrobiologie* 119: 35–53

Eldredge LG (2000) Non-indigenous freshwater fishes, amphibians, and crustaceans of the Pacific and Hawaiian islands. In: Sherley G (ed), Invasive Species in the Pacific: A Technical Review and Draft Regional Strategy. South Pacific Regional Environment Programme, Apia, Samoa, pp 173–190

Englund RA (1999) The impacts of introduced poeciliid fish and Odonata on the endemic *Megalagrion* (Odonata) damselflies of Oahu Island, Hawaii. *Journal of Insect Conservation* 3: 225–243, http://dx.doi.org/10.1023/A:1009651922486

Englund RA, Polhemus DA (2001) Evaluating the effects of introduced rainbow trout (*Oncorhynchus mykiss*) on native stream insects on Kauai Island, Hawaii. *Journal of Insect Conservation* 5: 265–281, http://dx.doi.org/10.1023/A:1013331525088

Engstrom-Heg R, Colesante RT, Silco E (1978) Rotenone tolerances of stream-bottom insects. *New York Fish and Game Journal* 25: 31–41

Finlayson BJ, Schnick RA, Cailteux RL, DeMong L, Horton WD, McClay W, Thompson CW, Tichacek GJ (2000) Rotenone Use in Fisheries Management: Administrative and Technical Guidelines Manual. American Fisheries Society, Bethesda, Maryland

Finlayson BJ, Schnick R, Skaar D, Anderson J, Demong L, Duffield D, Horton W, Steinkjer J (2010a) Planning and Standard Operating Procedures for the Use of Rotenone in Fish Management—Rotenone SOP Manual. American Fisheries Society, Bethesda, Maryland

Finlayson BJ, Somer WL, Vinson MR (2010b) Rotenone toxicity to rainbow trout and several mountain stream insects. *North American Journal of Fisheries Management* 30: 102–122, http://dx.doi.org/10.1577/M09-078.1

Finlayson BJ, Somer WL, Vinson MR (2012) Rotenone toxicity to rainbow trout and several mountain stream insects: Response to comment. *North American Journal of Fisheries Management* 32: 60–64, http://dx.doi.org/10.1080/02755947.2012.655846

Fisher JP (2007) Screening level risk analysis of previously unidentified rotenone formulation constituents associated with the treatment of Lake Davis. Report prepared by ENVIRON International Corporation for the California Department of Fish and Game, September 7, 2007

Font WF (2003) The global spread of parasites: What do Hawaiian streams tell us? *BioScience* 53: 1061–1967, http://dx.doi.org/10.1641/0006-3568(2003)053[1061:TGSOPW]2.0.CO;2

Fortes RD (2005) Review of techniques and practices in controlling tilapia populations and identification of methods that may have practical applications in Nauru including a national tilapia plan. Secretariat of the Pacific Community, Noumea Cedex, New Caledonia

Gehrke PC (2003) Preliminary assessment of oral rotenone baits for carp control in New South Wales. In: Munro R (ed), Managing Invasive Freshwater Fish in New Zealand, Proceedings of a Workshop. Hamilton, New Zealand, May 10–12, 2001. Department of Conservation, Wellington, New Zealand, pp 143–154

Gilmore RG, Hastings PA, Kulczycki GR, Jennison BL (1981) Crystalline rotenone as a selective fish toxin. *Florida Scientist* 44: 193–203

Guerrero RD, Guerrero LA, Garcia LL (1990) Use of indigenous plants as sources of fish toxicants for pond management in the Philippines. *Philippine Technology Journal* 15: 15–17

Hegen HE (1985) Use of rotenone and potassium permanganate in estuarine sampling. *North American Journal of Fisheries Management* 5: 500–502, http://dx.doi.org/10.1577/1548-8659(1985)5<500:UORAPP>2.0.CO;2

Holthuis LB (1973) Caridean shrimps found in land-locked saltwater pools at four Indo-West Pacific localities (Sinai Peninsula, Funafuti Atoll, Maui and Hawaii Islands), with the description of one new genus and four new species. *Zoologische Verhandelingen* 128: 1–48

Jones HA (1933) The rotenone content of derris root, cube root, and other plant materials. *Journal of the Washington Academy of Science* 23: 36–46

Kaplan EL, Meier P (1958) Nonparametric estimation from incomplete observations. *Journal of the American Statistical Association* 53: 188–193, http://dx.doi.org/10.1080/01621459.1958.10501452

Kolar CS, Courtenay WR, Nico LG (2010) Managing undesired and invading fishes. In: Hubert WA, Quist MC (eds), Inland Fisheries Management in North America, third edition. American Fisheries Society, Bethesda, Maryland, pp 213–259

Krumholz LA (1948) The use of rotenone in fisheries research. *Journal of Wildlife Management* 12: 305–317

Leonard JW (1939) Notes on the use of Derris as a fish poison. *Transactions of the American Fisheries Society* 68: 269–280, http://dx.doi.org/10.1577/1548-8659(1938)68[269:NOTUOD]2.0.CO;2

Lever C (1996) Naturalized Fishes of the World. Academic Press, London, 408 pp

Ling N (2003) Rotenone: A review of its toxicity and use for fisheries management. Science for Conservation Series 211, New Zealand Department of Conservation, Wellington, 40 pp

Lobel PS (1980) Invasion by the Mozambique tilapia (*Sarotherodon mossambicus*; Pisces; Cichlidae) of a Pacific atoll marine ecosystem. *Micronesica* 16: 349–355

Maciolek JA (1984) Exotic fishes in Hawaii and other islands of Oceania. In: Courtenay WR, Jr., Stauffer JR, Jr. (eds), Distribution, Biology, and Management of Exotic Fishes. The Johns Hopkins University Press, Baltimore, pp 131–161

MacKenzie RA, Bruland GL (2012) Nekton communities in Hawaiian coastal wetlands: The distribution and abundance of introduced fish species. *Estuaries and Coasts* 35: 212–226

Marking LL, Bills TD (1976) Toxicity of rotenone to fish in standardized laboratory tests. Investigations in Fish Control 72. US Department of the Interior, Fish and Wildlife Service, Washington, D.C.

McClay W (2005) Rotenone use in North America (1988-2002). *Fisheries* 30: 29–31, http://dx.doi.org/10.1577/1548-8446(2005)30[29:RUINA]2.0.CO;2

Meadows BS (1973) Toxicity of rotenone to some species of coarse fish and invertebrates. *Journal of Fish Biology* 5: 155–163, http://dx.doi.org/10.1111/j.1095-8649.1973.tb04444.x

Merrill ED (1943) Emergency food plants and poisonous plants of the Pacific. Technical monograph 10-420. United States Printing Office, Washington, D.C.

Metzger RJ, Shafland PL (1986) Use of detonating cord for sampling fish. *North American Journal of Fisheries Management* 6: 113–118, http://dx.doi.org/10.1577/1548-8659(1986)6<113:UODCFS>2.0.CO;2

Morin MP (1994) Hawaiian fishponds and endangered waterbirds on the Kona Coast. *Transactions of the Western Section of the Wildlife Society* 30: 66–71

Morin MP (1998) Endangered waterbird and wetland status, Kaloko-Honokohau National Historic Park, Hawai'i Island. Technical Report, 119. Cooperative National Park Resources Studies Unit, University of Hawaii at Manoa, Department of Botany, Manoa, Hawaii, 69 pp

Mundy BC (2005) Checklist of the fishes of the Hawaiian Archipelago. *Bishop Museum Bulletins in Zoology* 6: 1–704

Næss T (1991) Tolerance of marine calanoid resting eggs: Effects of freezing, desiccation and rotenone exposure - a field and laboratory study. *Marine Biology* 111: 455–459, http://dx.doi.org/10.1007/BF01319418

Næss T, Naas KE, Samuelsen OB (1991) Toxicity of rotenone to some potential predators on marine fish larvae: An experimental study. *Aquacultural Engineering* 10: 149–159, http://dx.doi.org/10.1016/0144-8609(91)90020-K

Nelson SG, Eldredge LG (1991) Distribution and status of introduced cichlid fishes of the genera *Oreochromis* and *Tilapia* in the islands of the South Pacific and Micronesia. *Asian Fisheries Science* 4: 11–22

Newman MC (2013) Quantitative Ecotoxicology, 2nd edition. CRC Press, Boca Raton, Florida

Newman MC, Aplin MS (1992) Enhancing toxicity data interpretation and prediction of ecological risk with survival time modeling: An illustration using sodium chloride toxicity to mosquitofish (*Gambusia holbrooki*). *Aquatic Toxicology* 23: 85–96, http://dx.doi.org/10.1016/0166-445X(92)90001-4

Newman MC, Dixon PM (1996) Ecologically meaningful estimates of lethal effect in individuals. In: Newman MC, Jagoe CH (eds), Ecotoxicology: A Hierarchical Treatment. CRC Lewis Publishers, Boca Raton, Florida, pp 225–253

Nico LG, Walsh SJ (2011) Nonindigenous freshwater fishes on tropical Pacific islands: A review of eradication efforts. In: Veitch CR, Clout MN, Towns DR (eds), Island Invasives: Eradication and Management. International Union for Conservation of Nature (IUCN), Gland, Switzerland, pp 97–107

Ogunsanya T, Durborow RM, Webster CD, Tidwell JH, Coyle S, Thompson K, Jarboe HH, Straus DL, Wang C, Huang L (2011) Toxicity of rotenone to giant river freshwater prawn *Macrobrachium rosenbergii. North American Journal of Aquaculture* 73: 159–163, http://dx.doi.org/10.1080/15222055.2011.568861

Oki DS, Tribble GW, Souza WR, Bolke EL (1999) Ground-water resources in Kaloko Honokohau National Historical Park, Island of Hawaii, and numerical simulation of the effects of groundwater withdrawals. U.S. Geological Survey, Honolulu, Hawaii. Water-Resources Investigations Report 99–4070, 49 pp

Pagán C (1948) The use of guppies in the toxicological assay of *Derris* and *Lonchocarpus* roots. *Journal of Economic Entomology* 41: 942–945

Parrish JD, Smith GC, Norris JE (1990) Resources of the marine waters of Kaloko-Honokohau National Historic Park. Cooperative National Park Resources Studies Unit, University of Hawaii at Manoa, Honolulu, Hawaii. Technical Report 74, 119 pp

Polhemus DA (1996) The orangeblack Hawaiian damselfly, *Megalagrion xanthomelas* (Odonata: Coenagrionidae):

Clarifying the current range of a threatened species. *Bishop Museum Occasional Paper* 45: 30–53

Quigley C (1956) Aboriginal fish poisons and the diffusion problem *American Anthropologist, New Series* 58: 508–525

Ranoemihardjo BS (1981) Nauru: Eradication of tilapia from fresh- and brackish-water lagoons and ponds with a view of promoting milkfish culture. Food and Agriculture Organization of the United Nations (FAO), Rome, Italy, 23 pp

Rickard PP, Cox PA (1986) Use of *Derris* as a fish poison in Guadalcanal, Solomon Islands. *Economic Botany* 40: 479–484, http://dx.doi.org/10.1007/BF02859661

Rowe-Rowe DT (1971) Rotenone tolerances of some freshwater fishes of Natal. *Progressive Fish Culturist* 33: 206–209, http://dx.doi.org/10.1577/1548-8640(1971)33[206:RTOSFF]2.0.CO;2

Russell DJ, Thuesen PA, Thomson FE (2012) Reproductive strategies of two invasive tilapia species *Oreochromis mossambicus* and *Tilapia mariae* in northern Australia. *Journal of Fish Biology* 80: 2176–2197, http://dx.doi.org/10.1111/j.1095-8649.2012.03267.x

Schnick RA (1974) A review of the literature on the use of rotenone in fisheries. U.S. Fish and Wildlife Service, Fish Control Laboratory, LaCrosse, Wisconsin

Schofield PJ, Nico LG (2007) Toxicity of 5% rotenone to nonindigenous Asian swamp eels. *North American Journal of Fisheries Management* 27: 453–459, http://dx.doi.org/10.1577/M05-206.1

Schofield PJ, Slack WT, Peterson MS, Gregoire DR (2007) Assessment and control of an invasive aquaculture species: An update on Nile tilapia (*Oreochromis niloticus*) in coastal Mississippi after Hurricane Katrina. *Southeastern Fishes Council Proceedings* 49: 9–15

See K, Godwin S, Menza C (2009) Nonindigenous and invasive species. In: Friedlander A, Keller K, Wedding L, Clarke A, Monaco M (eds), A Marine Biogeographic Assessment of the Northwestern Hawaiian Islands. Technical Memorandum NOS NCCOS 84. National Oceanic and Atmospheric Administration (NOAA), Silver Spring, Maryland, pp 275–290

Serns SL (1979) Effects of Pro-Noxfish® on the benthos and zooplankton of Bug Lake, Forest County, Wisconsin. *Journal of the American Water Resources Association* 15: 1385–1393, http://dx.doi.org/10.1111/j.1752-1688.1979.tb01137.x

Shimps EL, Rice JA, Osborne JA (2005) Hypoxia tolerance in two juvenile estuary-dependent fishes. *Journal of Experimental Marine Biology and Ecology* 325: 146–162, http://dx.doi.org/10.1016/j.jembe.2005.04.026

Spennemann DHR (2002) Traditional milkfish aquaculture in Nauru. *Aquaculture International* 10: 551–562, http://dx.doi.org/10.1023/A:1023900601000

St. Amant JA (1966) Addition of *Tilapia mossambica* Peters to the California fauna. *California Fish and Game* 52: 54–55

Stokes JFG (1921) Fish poisoning in the Hawaiian Islands with notes on the custom in southern Polynesia. *Occasional Papers of the B. P. Bishop Museum of Polynesian Ethnology and Natural History* 7: 210–233

Tourenq C, Shuriqi MK, Sawaf M, Smart E (2011) Removal of an introduced tilapia species from a wadi in the United Arab Emirates. *The Invasive Species Bulletin* 31: 60–69

USEPA (2007) Reregistration eligibility decision for rotenone. EPA 738-R-07-007 March 2007. U.S. Environmental Protection Agency, Washington, D.C., 44 pp

USFWS (2011) Recovery plan for Hawaiian waterbirds, second revision. U.S. Fish and Wildlife Service, Portland, Oregon, xx + 233 pp

USNPS (2013) Kaloko-Honokohau cultural center: Environmental assessment. U.S. National Park Service, Department of the Interior, Kaloko-Honokohau National Historical Park, Kailua-Kona, Hawai'i, 110 pp

Vasquez ME, Rinderneck J, Newman J, McMillin S, Finlayson B, Mekebri A, Crane D, Tjeerdema RS (2012) Rotenone formulation fate in Lake Davis following the 2007 treatment. *Environmental Toxicology and Chemistry* 31: 1032–1041, http://dx.doi.org/10.1002/etc.1795

Vinson MR, Dinger EC, Vinson DK (2010) Piscicides and invertebrates: After 70 years, does anyone really know? *Fisheries* 35: 61–71, http://dx.doi.org/10.1577/1548-8446-35.2.61

Vitousek S, Barbee MM, Fletcher CH, Richmond BM, Genz AS (2009) Puʻukoholā Heiau National Historic Site and Kaloko Honokōhau Historical Park, Big Island of Hawai'i: Coastal hazard analysis report. U.S. National Park Service, Geological Resources Division, Fort Collins, Colorado. Natural Resource Technical Report NPS/NRPC/NRTR/GRD-2010/387, 105 pp

Watkins WD, Tarter DC (1974) Acute toxicity of rotenone on the naiadal stage of the dragonfly, *Basiaeschna janata* Say, under laboratory conditions. *Proceedings of the West Virginia Academy of Science* 46: 141–145

Wilson CA (1990) Extensive aquaculture of red drum in the southern United States. In: Chamberlain GW, Miget RJ, Haby MG (eds), Red Drum Aquaculture. Texas A&M Sea Grant Publication 90-603, Galveston, Texas, pp 154–164

Wingard CJ, Swainson CJ (1992) Ventilatory responses of four marine teleosts to acute rotenone exposure. *Journal of Applied Ichthyology* 8: 132–142, http://dx.doi.org/10.1111/j.1439-0426.1992.tb00675.x

Woodford DJ, Barber-James HM, Bellingan TA, Day JA, de Moor FC, Gouws J, Weyl OLF (2013) Immediate impact of piscicide operations on a Cape Floristic Region aquatic insect assemblage: A lesser of two evils? *Journal of Insect Conservation* 17: 959–973, http://dx.doi.org/10.1007/s10841-013-9578-4

Yamamoto MN, Tagawa AW (2000) Hawai'i's Native and Exotic Freshwater Animals. Mutual Publishing, Honolulu, Hawaii, 200 pp

Zhao Y, Newman MC (2004) Shortcomings of the laboratory-derived median lethal concentration for predicting mortality in field populations: Exposure duration and latent mortality. *Environmental Toxicology and Chemistry* 23: 2147–2153, http://dx.doi.org/10.1897/03-557