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Determining the minimum effective dose of rotenone for eradication of alien smallmouth bass *Micropterus dolomieu* from a South African river

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In February 2012 the Rondegat River, in the Cape Floristic Region, was the first river in South Africa where the piscicide rotenone was used to remove an alien invasive fish, smallmouth bass *Micropterus dolomieu*. In preparation for this treatment, the sensitivity of smallmouth bass to various concentrations of the rotenone formulation CFT Legumine (5% active rotenone) was evaluated a week prior to treatment using standard toxicity tests to determine the minimum effective dose (MED) that would result in 100% mortality after exposure for 4 h. The MED was 0.0125 mg l^{-1} rotenone. Adverse effects, including erratic swimming, loss of equilibrium and death, occurred in a dose-dependent manner with smaller fish responding faster than larger ones. Standard operating procedures for the use of rotenone recommend treatment at a minimum of twice the calculated MED. Given the uncertainty associated with rotenone losses through hydrolysis and photolysis under field conditions, and the possible occurrence of smallmouth bass more tolerant than those tested, a concentration of twice the recommended treatment dose (0.050 mg l^{-1} rotenone) was finally used to treat the Rondegat River for a duration of 6 h.

Keywords: behaviour effects, biodiversity conservation, effective concentration, mortality, rotenone toxicity

Introduction

The Cape Floristic Region (CFR) of South Africa is an area of high fish diversity and endemism (Linder et al. 2010) where the primary threat to many of the endangered native fishes is predation by alien fish species (Tweddle et al. 2009). The removal of alien fishes from rivers containing threatened endemic freshwater fishes is therefore a conservation priority. Piscicides are commonly used to eradicate undesirable fishes (Lintermans 2000, Chadderton et al. 2003, Ling 2003, Britton and Brazier 2006, Finlayson et al. 2010, Vasquez et al. 2012) and, in South Africa, treatment with the piscicide rotenone has been identified as a potential biodiversity restoration tool for selected rivers in the CFR (Impson 2007). As a result, the Western Cape provincial conservation agency CapeNature commissioned an extensive environmental impact assessment (EIA) on the use of rotenone for the rehabilitation of four CFR rivers (Marr et al. 2012). Based on the positive outcome of this EIA process, the eradication of smallmouth bass *Micropterus dolomieu* (Lacepède 1802) from the middle section of the Rondegat River was chosen as a pilot project for using the piscicide rotenone for alien fish eradication in South Africa (Marr et al. 2012).

Rotenone is a naturally occurring chemical derived from the roots of several plants in the genera *Lonchocarpus* and *Derris* (Leguminosae) (Brooks and Price 1961). A target organ for rotenone toxicity has not yet been identified, although it is known that rotenone inhibits oxidative phosphorylation by blocking respiration in the mitochondria (Hollingworth 2001). Following exposure, rotenone is metabolised by the NADPH enzyme system in fish (Fukami et al. 1969). Rotenone exhibits selective toxicity and, at relatively low concentrations (<0.25 mg l^{-1} active ingredient), it is highly effective at killing most fish species but is non-toxic to plants, birds and mammals at the concentrations used (Johnson and Finley 1980, Cheng and Farrell 2007). It is non-persistent in the aquatic environment and is degraded by photolysis, hydrolysis and metabolism (Engstrom-Heg and Colesante 1979, Sariaslani et al. 1984, USEPA 2007, Finlayson et al. 2010). Rotenone, however, poses a threat to other gill-breathing organisms such as macroinvertebrates and larval amphibians (Johnson and Finley 1980, Blakeley et al. 2005, Billmann et al. 2011) and possible negative impacts of rotenone on these non-target species has become a contentious issue (Finlayson et al. 2005, 2009, Vinson et al. 2010). It is therefore important that treatments utilise the lowest efficacious concentrations of rotenone to accomplish the objective. Rach et al. (2009) showed that fish exposed to sublethal concentrations of rotenone can recover, which illustrated the risk of using low and thus ineffective treatment concentrations.

To determine appropriate rotenone concentrations, Finlayson et al. (2010) recommend that a bioassay be conducted before a river treatment to determine the minimum effective dose (MED) that will result in the desired objective. For eradication, the MED is usually calculated as the minimum dose that results in 100% mortality within a

specific exposure time frame. Standard operating procedures (SOP) for the use of rotenone recommend that bioassay tests are conducted using (1) the target fish species, (2) the rotenone from the stock to be used and (3) the water from the river that will be treated (Finlayson et al. 2010). The reasons for this are the variability in the response of the target species to rotenone, the influence of the local water quality conditions on the efficacy of the rotenone and the variability of the active rotenone in formulations that have been in storage, as rotenone degrades over time. The objective of the current study was therefore to determine the MED for smallmouth bass in Rondegat River water during short-term exposure of 4–8 h using CFT Legumine, containing 5% active ingredient, i.e. the rotenone stock that was to be used in the river treatment.

Materials and methods

This study was carried out in February 2012 using the SOP manual for the use of rotenone in fish management (Finlayson et al. 2010) and the Fish Acute Toxicity Test Guideline 203 (OECD 1992) for guidance.

Experimental animals

Wild-caught smallmouth bass of a similar size (mass 48.2 ± 16.6 g, total length 165.6 ± 18.6 mm) from the proposed treatment zone of the Rondegat River were used for all toxicity tests. After collection, fish were held in an aerated 500-litre holding bin containing water from the river and acclimated for 24 h prior to testing. Fish were not fed during the acclimation period and a 30% water change was done daily for the holding bin. The size of the smallmouth bass reflected the population structure of this species in the river (Weyl et al. 2013). Each treatment group consisted of five randomly selected fish in the size class stated. In addition, to assess for potential behaviour effects of rotenone toxicity related to body size, one larger fish (mass 80.2 ± 14.1 g, total length 199.6 ± 10.3 mm) and one smaller fish (mass 5.2 ± 1.6 g, total length 76 ± 8.2 mm) were added to each group, unless stated otherwise.

Experimental design

The static toxicity test was conducted in identical 500-litre tanks using river water, and treatments and fish were randomly assigned to a tank. Smallmouth bass were stocked at densities lower than the maximum density of 1.0 g fish l^{-1} prescribed by standard toxicity testing guidelines (OECD 1992). To simulate natural conditions, the test was conducted outdoors in the shade at ambient temperature, resulting in a mean water temperature of 25 ± 1.9 °C. All tanks were aerated during the test for the duration of the experiment and temperature, pH and conductivity were measured at the onset of each experiment. Measured values were temperature 22.3–28.0 °C, pH 6.3–7.1 and conductivity 28–44 µS cm^{-1}. Dissolved oxygen was not measured, but was expected to be >60% saturation as all tanks were constantly aerated and fish were stocked at low densities. The commercial piscicide formulation CFT Legumine (5% active rotenone), registered in the USA (EPA registration number 75338-2), was used. Smallmouth bass were exposed to three concentrations (0.025, 0.50 and 2.0 mg l^{-1}) of CFT Legumine, yielding 0.0125, 0.025 and 0.100 mg l^{-1} rotenone, respectively. CFT Legumine was diluted into a stock solution that was added into the exposure tanks to obtain the selected exposure concentrations. A new stock solution was made for every replicate, and stock solution not used within 6 h was discarded.

All rotenone treatments were tested in triplicate, with a minimum of five smallmouth bass in each treatment (n = 15 per concentration). Each replicate had a control group, also consisting of five fish. Smallmouth bass were observed constantly for the first hour of the test, and then at 15-minute intervals until the end of the test. Mortality was defined as the loss of all opercular movement (OECD 1992) for a period of at least 5 min.

Statistical analysis

A number of behaviour traits associated with rotenone toxicity were statistically compared among the three treatment groups, using a Kruskal–Wallis analysis of variance. These traits were not observed in the control group. The traits were the time from initiation of rotenone exposure (t_0) to (1) when the first fish breached the water surface, (2) when the first fish showed its first sign of losing equilibrium and (3) when the first fish ceased opercular movement (i.e. time of death). Statistical analysis was done only on groups from the medium size class, as statistical analysis was not possible on the smaller and larger groups of fish because of small sample sizes.

Results

Mortality and general behaviour changes

All treated fish died during exposure, whereas no mortality was observed in any of the control fish. Similar behaviour effects, induced in a time- and dose-dependent manner, were observed during all treatments. In the highest rotenone treatment concentration (0.100 mg l^{-1}), fish were schooling and showing signs of being agitated and hyperactive within the first 5 min of initiating the exposure, and severe behaviour aberrations, including spiralling, darting and loss of equilibrium, had manifested within 10 min exposure time. Fish in the 0.025 mg l^{-1} rotenone exposure exhibited similar symptoms, but this only manifested after 10 min of exposure, whereas the aberrant swimming behaviour only became apparent after 20 min of being exposed to rotenone. The onset of toxicant-induced effects in the 0.0125 mg l^{-1} rotenone exposure was the slowest of all exposure groups, with fish becoming agitated or showing an increase in swimming behaviour only after 30–40 min. Spiralling and darting behaviour in these groups was observed after 40–45 min. Following the manifestation of aberrant swimming behaviour, all fish in all exposure groups lost equilibrium completely and sank to the bottom of the tanks, where normal opercular movement continued for some time, after which movement slowed and became irregular until the point of death.

Specific endpoints

Specific endpoints, namely the time when the first fish breached the water surface, the time to when the first fish lost equilibrium, and the time to when the first fish ceased

all opercular movement, were observed in all treatments. Fish exposed to the highest concentration breached, lost equilibrium and ceased opercular movement (died) faster than fish exposed to lower concentrations ($p < 0.05$). A clear dose–time response was observed for all selected behaviour endpoints (Figure 1).

Body-size-related effects

Given the very small sample size for the larger and smaller fish, statistical analysis of these size class data was not possible. However, a body-size-related effect was observed consistently for all behaviours throughout all replicates in all three concentrations. Smaller fish were affected first, irrespective of treatment concentration (Table 1).

Discussion

Within the rotenone concentration range tested (0.0125–0.100 mg l^{-1}), the onset of symptoms was typically rapid at the two higher concentrations and slower in the lowest concentration. This is consistent with the results obtained using other freshwater fish species, including common carp *Cyprinus carpio* (Fajt and Grizzle 1998), northern snakeheads *Channa argus* (Lazur et al. 2006) and rainbow trout *Oncorhynchus mykiss* (Cheng and Farrell 2007). These studies used rotenone concentrations ranging from 0.075 to 0.3 mg l^{-1} for *C. argus*, 0.1 mg l^{-1} for *C. carpio* and 0.005–0.0075 mg l^{-1} for *O. mykiss*. In spite of low sample sizes, body size covaried with the onset of behavioural endpoints as well as time to death, which provides evidence that smaller fish are more sensitive to exposure than larger fish. Similar results were reported for several fish species, including largemouth bass *Micropterus salmoides* and spotted bass *Micropterus punctulatus* during both laboratory and field exposures (Hester 1959, Rowe-Rowe 1971, Chadderton et al. 2003). Brown et al. (2011) found a significant but weak correlation between rainbow trout size and time to death for rotenone exposures. Smaller fish appeared to be affected more quickly, but they did not consistently die before the large fish, and less than 21% of the observed variation could be explained by body size.

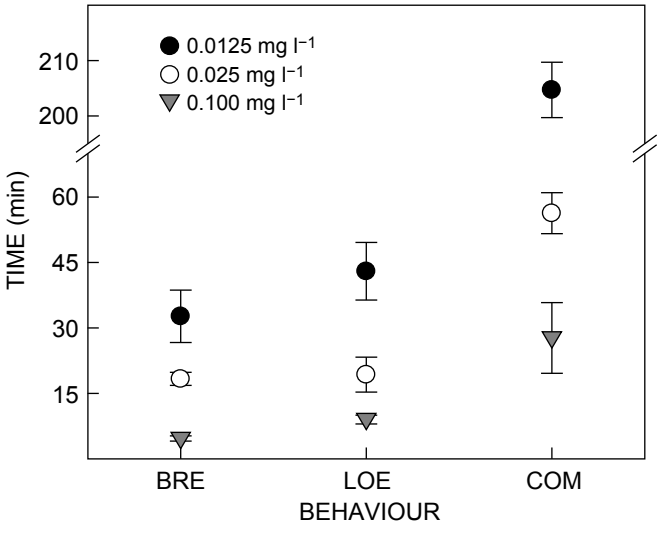

Figure 1: Relationship between the time to onset of selected behaviour endpoints and the rotenone concentrations tested. BRE = Breaching of water surface, LOE = loss of equilibrium, COM = cessation of opercular movement. Error bars represent SD

The reason for the increased sensitivity of smaller, probably younger, individuals in the present study can possibly be attributed to the relative gill surface area and gill surface area to body volume ratio being larger in small fish than in larger fish (Oikawa and Itazawa 1985, Randall et al. 1996). As the gills are the primary route of uptake of the rotenone (Oberg 1965), the toxicant will potentially build up to toxic levels and reach the target enzymes faster in smaller animals, thus resulting in a faster onset of symptoms leading to death. It is also possible that younger fish have less well-developed detoxification mechanisms in the liver and are therefore not as efficient in deactivating rotenone as adult individuals. This was proposed by Chandrasekara and Pathiratne (2007) to explain body-size-related differences in sensitivity of Nile tilapia *Oreochromis niloticus* to organophosphates. It is also well known that younger animals are in most cases more sensitive to toxicants than older or mature animals (Rozman et al. 2001). Younger and smaller fish respond faster than older and larger fish, but this does not necessarily translate into lower LC_{50} values for younger fish, and this may make little to no difference on the dose required when treating lakes or streams with exposure times of ≥6 h.

Marking and Bills (1976) reported 24 h and 96 h LC_{50} values for *M. dolomieu* of 0.093 and 0.079 mg l^{-1} rotenone, respectively, when exposed to the commercial piscicide formulation Noxfish (5% active ingredient). In the current study, the lowest concentration of rotenone tested that resulted in 100% smallmouth bass mortality was 0.0125 mg l^{-1}, and this can be used to estimate the MED for a 4 h exposure. This concentration would theoretically result in complete mortality in 4 h, provided conditions in the toxicity tests were similar to those in the Rondegat River. Experimental conditions differed from the expected field situation in that fish size was standardised and there was no direct ultraviolet (UV) radiation.

Rotenone has a photolysis half-life of 1.4 h (Spare 1982) to 8.2 h (Draper 2002). High-intensity UV radiation, typical of the Cederberg in summer (Janse van Rensburg 2009),

Table 1: Mean (±SD) response times of different *Micropterus dolomieu* size classes at the various rotenone concentrations tested. BRE = Breaching of water surface, LOE = loss of equilibrium, COM = cessation of opercular movement, *n* = number of treatments

Size class	Concentration (mg l^{-1})	BRE (min)	LOE (min)	COM (min)	*n*
Small	0.0125	17 ± 2.8	26 ± 5.7	118 ± 9.9	2
Small	0.025	12.3 ± 1.2	15.7 ± 2.5	40 ± 2.6	3
Small	0.100	3	7	14	1
Medium	0.0125	32.7 ± 6	43 ± 6.6	204.7 ± 5	3
Medium	0.025	18.3 ± 1.5	19.3 ± 4	56.3 ± 4.7	3
Medium	0.100	4.7 ± 0.6	9 ± 1	27.7 ± 8.1	3
Large	0.0125	59.3 ± 16.6	73 ± 17.1	208 ± 3.5	3
Large	0.025	23	26	68	1
Large	0.100	12	14	35	1

and the possibility of larger, and possibly less sensitive, smallmouth bass indicate that a concentration higher than 0.0125 mg l^{-1} rotenone for 4 h may be necessary for successful eradication of this species from the Rondegat River. Given that the success of the Rondegat rehabilitation project depended on the complete removal of the target species from the river section, a rotenone concentration higher than 0.0125 mg l^{-1} would probably be required to ensure eradication of the smallmouth bass. Finlayson et al. (2010) recommended treating at a concentration of at least twice the MED to ensure eradication, resulting in a minimum rotenone concentration of 0.025 mg l^{-1} being required for smallmouth bass eradication in the Rondegat River.

Standard operating procedures (Finlayson et al. 2010) and the label for CFT Legumine recommend a treatment time of 4–8 h to eradicate fish from streams. Given concerns regarding UV radiation degrading rotenone, and the possible presence of more tolerant fish, the Rondegat River was subsequently treated at a rotenone concentration of 0.050 mg l^{-1} (i.e. four times the MED) for 6 h (BJ Finlayson, California Department of Fish and Game, pers. comm.). The 0.050 mg l^{-1} rotenone concentration was within the range recommended to minimise impacts to non-target aquatic invertebrates during the eradication of rainbow trout, which are similar in sensitivity to smallmouth bass (Marking and Bills 1976), in mountain streams (Finlayson et al. 2009). In the Rondegat River the treatment resulted in the mortality of target as well as non-target fish species, and a post-treatment survey demonstrated that fish numbers were below detectable levels (Weyl et al. 2013). The effectiveness of rotenone applications to streams and rivers is affected by a variety of factors, and therefore it is advisable to conduct multiple treatments to ensure complete removal of target fish from the project area. This is accomplished by conducting applications until no more target fish are removed during a subsequent treatment. As recommended by the SOP (Finlayson et al. 2010), CapeNature has scheduled a follow-up treatment in March 2013 (one year after the initial treatment).

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