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INVASION NOTE

# Unexpected survival of sharptooth catfish *Clarias gariepinus* (Burchell 1822) during acute rotenone toxicity trials will complicate management of invasions

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**Abstract** The sharptooth catfish *Clarias gariepinus* is an emerging global invader for which control strategies might include the use of piscicides such as rotenone. Experimental exposure demonstrated that *C. gariepinus* was less susceptible to rotenone than most other fish species, with unexpected survival observed at rotenone concentrations of 87.5 and 100 μg $L^{-1}$. *C. gariepinus* were also observed exhibiting avoidance behaviour to rotenone treated water and were found to be capable of recovering from rotenone exposure. As such, effective eradication might not be attainable even at a dose exceeding 100 μg $L^{-1}$ with exposures of longer than 24 h. This exposure scenario may pose an unacceptable risk to non-target fauna and highlights the difficulty associated with managing current and future invasions.

**Keywords** Acute toxicity · Behaviour effects · Biodiversity restoration · Global invader · Invasions · Rotenone

## Introduction

The African sharptooth catfish *Clarias gariepinus* is an emerging global invader that has been introduced into 37 countries for aquaculture purposes (Weyl et al. 2016). The same biological traits that make *C. gariepinus* desirable as an aquaculture species (e.g., fast growth, early maturity and high fecundity) have enabled its establishment in the wild in many countries including Brazil, China, India, Taiwan and South Africa (Weyl et al. 2016). As a result of the high potential impact of this large predator on native biota (Alexander et al. 2014; Ellender et al. 2015; Weyl et al.

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2016) invasions will require management. As invasive fishes generally have life history traits that facilitate the rapid colonisation of environments from small founder populations, complete eradication via dewatering or the use of piscicides are considered the only viable methods for their eradication (Finlayson et al. 2010). Clariid catfishes have, however, evolved a supra-branchial organ which facilitates air-breathing in low oxygen environments and allows them to survive even complete desiccation and leave the aquatic environment and "walk" using pectoral fins (Bruton 1979). This makes dewatering an unlikely eradication option and may complicate the use of piscicides as a population management tool.

Rotenone, a naturally occurring botanical compound ($C_{23}H_{22}O_6$) which exerts a toxic effect by disrupting normal aerobic cellular respiration in gill-breathing organisms by blocking mitochondrial electron transport (Singer and Ramsay 1994), has been successfully used for managing other alien fishes for biodiversity restoration purposes in Australia (Rayner and Creese 2006), New Zealand (Pham et al. 2013), Northern America (Finlayson et al. 2009), Europe (Allen et al. 2006) and South Africa (Weyl et al. 2014). As the use of rotenone is an accepted management strategy for alien fish removal in South Africa's Cape Fold Ecoregion (Weyl et al. 2014) where *C. gariepinus* have recently spread into rivers that contain endemic and highly threatened freshwater fish fauna (Ellender et al. 2015), we assessed its suitability as a management tool for this species. To do this, we conducted an experiment to investigate the acute toxicity of rotenone and determine concentrations that would result in 100% mortality [minimum effective dose (MED)] of *C. gariepinus* during typical treatment durations (up to 24 h; Slabbert et al. 2014) and assessed whether *C. gariepinus* would actively avoid exposure (e.g., by air breathing and leaving the water) and/or recover after exposure.

## Experimental design

Experiments were conducted using fish that were of comparable size to those that typically invade headwater streams (Ellender et al. 2015). Experiments were conducted on hatchery-reared catfish measuring (mean ± standard deviation) 349 ± 19 mm TL and weighing 309 ± 55 g in identical 500 L circular tanks containing matured tap water. To allow for the assessment of behavioral responses an avoidance platform was constructed using a plastic laundry bin covered in shade cloth. This was placed, partially submerged, in the exposure tank to present the fish with the option of moving completely out of the water. Prior to the experiment, four fish were randomly assigned to each tank resulting in a biomass of approximately 3 g fish per litre. Fish were acclimated for 24 h prior to testing and not fed during the acclimation period and the duration of the experiment. Temperature (range = 20–22.1 °C), pH (6.1–7.5) and conductivity (40.3–69.6 μS $cm^{-1}$) were measured at the onset of each experiment using an Aqualytic AL15 water quality meter (Aqualytic, Germany). As *C. gariepinus* are capable of air breathing, tanks were not aerated during the experiments.

Experiments to determine acute toxicity were conducted using six exposure concentrations (0, 50, 62.5, 75, 87.5 and 100 μg $L^{-1}$) of CFT Legumine® for a 24 h period. These concentrations are within the United States labeling recommendations for removing common carp *Cyprinus carpio* and bullheads *Ameiurus* spp. from ponds (Finlayson et al. 2010). Each concentration was tested on four replicates of four fish each (20 fish in total per treatment). Mortality was defined as a lack of response to touch coupled with the loss of all opercular movement for a period of at least two minutes (OECD 1992).

During exposure, fish behaviour was observed constantly for the first 90 min of the experiment and then again at six and 24 h of rotenone exposure. The time to the onset of some behaviour endpoints associated with rotenone exposure were investigated during the study. These behavior endpoints were the time from initiation of rotenone exposure ($t_0$) to: (a) when the first fish attempted to move onto an avoidance platform; (b) when the first fish was seen hanging vertically in the water column; (c) when the first fish was lying on the side of the body on the bottom of the tank and (d) when they first exhibited darting and/or spiraling behaviour. Air gulping was not included as this behaviour was routinely observed in the stock fish as well as in the control fish. Any fish surviving to the end of the 24 h exposure period, were moved to clean water to determine if a moribund state resulted in death or if recovery was possible. Fish in recovery tanks were inspected after 24 h in clean water.

## Results and discussion

### Mortality

None of the fish in the control groups died, nor did they exhibit any of the behavioral endpoints associated with rotenone exposure. Fish behavior in the control tanks was characterised by slow swimming and intermittent periods of resting when the fish were suspended diagonally in the water column, with the tail on the bottom of the tank and the snout facing forward. Behaviour of fish in rotenone treatments was characterised by slow swimming in the early stages of exposure, similar to the control fish, after which the fish became less active and ceased swimming altogether, often crowding at the side of the tank. Activity levels increased 10–15 min into the rotenone exposure and were followed by avoidance response (i.e. movement onto the avoidance platform), loss of equilibrium (fish hanging vertical in water column and later motionless on the bottom of the tank), darting and spiraling and finally death.

Comparisons of behavioral endpoints associated with rotenone toxicity (Kruskal–Wallis ANOVA, multiple comparison of mean ranks for all groups, Fig. 1; Table 1) and mortality data (Table 2) were inconsistent with the strong relationship between treatment exposure concentration and mortality reported for most fishes (e.g., Allen et al. 2006; Rach et al. 2009; Jordaan and Weyl 2013). Although the mean time to onset of symptoms associated with toxicity did show a dose–response with slower responses at lower concentrations (Fig. 1), the survival of one fish each in the two highest concentrations (87.5 and 100 μg $L^{-1}$) at 24 h was unexpected, as 100% mortality was observed at 75 μg $L^{-1}$ treatment (Table 2).

In addition, while survival differed significantly between treatment groups (Kruskal–Wallis ANOVA, Conover's-test for multiple comparisons of independent samples) at the 6 h ($p = 0.004$) and 24 h ($p = 0.007$) exposure times, this was not consistent with treatment concentration, as there was no clear relationship between exposure concentration and mortality (Table 2). For example, after an exposure of 6 h, survival in the 62.5 μg $L^{-1}$ was significantly lower than that at the lower 50 μg $L^{-1}$ concentration (Table 2). Similarly, after an exposure of 24 h, unexpected survival was observed in fishes exposed to concentrations of 87.5 and 100 μg $L^{-1}$ of active rotenone. This apparent lack of a linear dose–response relationship was not expected and suggests that there is considerable individual variation with regard to sensitivity to rotenone.

### Avoidance behaviour

While fish in the control groups ignored the avoidance platform, some individual fish within all rotenone treatments were observed approaching and

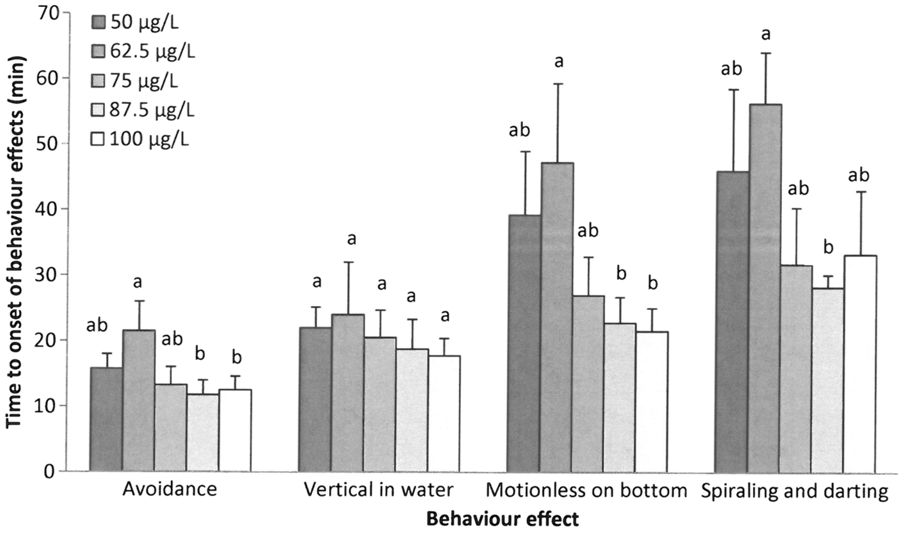


**Fig. 1** *Bar chart* indicating the mean time to onset of behaviour effects associated with rotenone toxicity at various exposure concentrations. Different *letters* denote statistical differences ($p < 0.05$) between treatment groups. *Error bars* denote standard deviations

**Table 1** Results of a Kruskal–Wallis ANOVA for time to onset of behaviour endpoints in *C. gariepinus* following exposure to rotenone

| Behaviour endpoint | Kruskal–Wallis Chi squared | df | *p* value |
|---|---|---|---|
| Avoidance | 12.269 | 4 | 0.016 |
| Vertical in water | 3.704 | 4 | 0.448 |
| Motionless on bottom | 14.024 | 4 | 0.007 |
| Spiralling and darting | 11.070 | 4 | 0.026 |

**Table 2** Mortality of *C. gariepinus* following exposure to various concentrations of rotenone

| Exposure concentration (μg $L^{-1}$) | No of fish exposed | Mortality at 6 h (%) | Mortality at 24 h (%) |
|---|---|---|---|
| 0 | 20 | 0 (0%) | 0 (0%) |
| 50.0 | 16 | 12 (75%) | 13 (81%) |
| 62.5 | 16 | 8 (50%)* | 11 (69%) |
| 75.0 | 16 | 12 (75%) | 16 (100%)* |
| 87.5 | 16 | 14 (88%) | 15 (94%)* |
| 100.0 | 16 | 15 (94%) | 15 (94%)* |

All groups were different from the control and significant differences between treatment groups at $p < 0.05$ is indicated by *

investigating the avoidance platform or moving onto the platform with >50% of the anterior portion of the body out of the water. In some cases the fish remained on the platform and out of the water for several minutes. During all exposures, some fish also attempted to move behind and under the platform, a behaviour not observed in control fish. Avoidance behaviour by air-breathing fishes has not previously been reported. Lazur et al. (2006) failed to demonstrate an avoidance response during an evaluation of the efficacy of rotenone to northern snakehead catfish *Channa argus*. This is in contrast with the present study, where an avoidance response (leaving the water) was observed in all rotenone treatment groups but not in any of the control fish. The presence of avoidance behaviour, coupled with the ability to air-breathe, is likely to pose a challenge when using rotenone for the management of this species as active avoidance by moving out of the water during treatments could theoretically decrease the actual exposure time and thus affect the uptake of the rotenone.

Post exposure recovery

Surviving individuals from all treatments except the 100 μg $L^{-1}$ were moved to clean water at the end of the 24 h rotenone exposure. Despite these individuals appearing moribund, recovery was observed for all fish after 24 h in clean water with the exposed fish showing a strong avoidance response to being touched with a blunt-ended aluminum rod, comparable to that of the control fish. While the long-term survival of these fish was not assessed in the present study, a cautionary approach should be employed when considering the implications of recovery to rotenone exposure by *C. gariepinus*.

## Synthesis and conclusion

*Clarias gariepinus* seem to be less sensitive to rotenone than many other fishes and there seems to be high levels of variability between individuals with regard to their susceptibility of rotenone. High individual variation and individual fish exceptionally resistant to rotenone has been reported (Meyer 1966) which highlights the need to apply a concentration greater than which is adequate in the laboratory to ensure a complete fish kill in a field situation. Furthermore, water quality parameters, mainly dissolved oxygen and temperature, may influence the behaviour of the target species (avoidance behaviour and air breathing) and thus impact on the efficacy of the treatment. Current US labelling permits rotenone treatments in the range of 5–50 μg $L^{-1}$ (depending on the sensitivity of the target species), increasing this to 100 μg $L^{-1}$ for resistant species such as bullhead catfish and carp and allowing a maximum treatment concentration of 200 μg $L^{-1}$ for resistant species in organic ponds only (Finlayson et al. 2010). Long exposure times and a MED of 100 μg $L^{-1}$ or higher will complicate treatments particularly in lotic environments. Standardised times for river treatments are generally short (4–12 h, Rach et al. 2009; Finlayson et al. 2010) and consistent chemical concentrations are hard to achieve. Slabbert et al. (2014) for example, monitored active rotenone concentrations during a river treatment in South Africa, and demonstrated that the actual rotenone concentration was consistently below the nominal treatment concentration. As a result, standard operating guidelines (Finlayson et al. 2010) recommend treatment concentrations at 200% of the MED which, in this case, would require treatment concentrations of 200 μg $L^{-1}$. Even then, the observed avoidance behaviour in rotenone treated water and recovery of moribund individuals following their transfer to clean water is likely to compromise eradication attempts. In addition, the study did not assess rotenone sensitivity of large specimens, an important consideration given that the species attains very large sizes (http://www.fishbase.org/summary/1934). Careful consideration is, therefore, needed for the use of rotenone for the eradication of *C. gariepinus* from the wild given that particularly high concentrations of rotenone will be necessary in this regard. A scenario of increased treatment time and high treatment doses provides a high-risk situation for non-target fauna. This requires a quantification of the trade-off between the impacts of the invader in the long term versus the short-medium term severe impacts of rotenone treatments. Given the results of the present study (unexpected survival at relatively high treatment doses, post treatment recovery and avoidance response), the management of existing *C. gariepinus* invasions is likely to be complex and invasions into novel ecosystems should be prevented.

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