Testing the efficacy of rotenone as a piscicide for New Zealand pest fish species

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ABSTRACT

The first South Island populations of *Gambusia affinis* and Koi carp were discovered in the Nelson region in May and July (2000), respectively. A delimitation survey using a standardised rapid sampling protocol was undertaken to determine how widespread these species were within the Nelson/Marlborough region. A total of 219 ponds and 54 waterways were surveyed over the warmer summer months and *Gambusia* were located in a further 19 farm ponds, and koi in one. In addition, illegally liberated populations of rudd, tench and red finned perch were also detected. These latter species were previously absent from the region. Survey data suggested that *Gambusia* and koi were restricted to farm ponds, although there was potential that they could spread from these into natural waterways. Under a pesticides board experimental licence, rotenone was applied to 17 ponds in an effort to eradicate these founding populations. Initial observations suggest that this was successful in killing all koi and *Gambusia* within these ponds. We discuss the results of these trials, efficacy of the application techniques used, rate of breakdown and variable responses of the different fish poisoned. Results of preliminary trials to resuscitate non-target fish species are also reported.

1. INTRODUCTION

Rotenone has been used extensively by fisheries managers in North America since the 1930s (Finlayson et al. 2000) and is recognised as the most environmentally benign of the commonly used fish poisons (Ling 2003). Its use in New Zealand has been limited to a few small water bodies with the only significant reported application being the 1981 removal of fish from Lake Parkinson, a small 1.9-ha dune lake south of Auckland (Rowe & Champion 1994). Rotenone has also been used extensively as a marine fish sampling tool, principally applied by taxonomist from the Museum of New Zealand (Paulin & Roberts 1993).

The first South Island populations of *Gambusia affinis* and koi carp (*Cyprinus carpio*) were discovered in the Nelson region in May and July (2000), respectively. A delimitation survey of 219 water bodies and 54 waterways using a standardised rapid sampling protocol was undertaken to determine how widespread these species were within the Nelson/Marlborough region. This survey located a further 19 *Gambusia* populations, one of which also contained koi carp. In addition, rudd (*Scardinus erythrophthalmus*), tench (*Tinca tinca*) and red finned perch (*Perca fluviatilus*) were also discovered. All these species were previously absent from the region and had been illegally introduced (details reported in Shaw & Studholme 2001). *Gambusia* and koi were largely restricted to small artificial waterbodies (with 0.05–4.4 ha surface area) and in order to prevent their establishment into larger natural waterways the Department of Conservation (DOC) decided to attempt to eradicate these fish using rotenone (Shaw & Studholme 2001).

However, rotenone was not registered as a fish poison in New Zealand. The eradication attempts were therefore actioned under an experimental use permit granted by the Pesticides Board, under the auspices of testing the efficacy of rotenone as a piscicide in New Zealand. The permit required that all operations be monitored and the effects and persistence of the poison in the application environments were documented.

This paper reports the results of these trials, specifically the efficacy of the two application techniques (fire pump and helicopter boom-sprayer) to spread rotenone at the desired concentration and whether they achieved eradication. In addition, we report on the temporal and spatial variation in the rates of breakdown of rotenone at different ponds, the response of target and non-target fish species to rotenone poisoning and the effectiveness of initial revival trials.

2. METHODS

2.1 Measuring rotenone

Water samples were collected 3 h post spraying, at five ponds where a fire pump and hoses were used to apply the poison, and at the one site where a helicopter mounted boom-sprayer was used. At all ponds, two samples were taken from the edge. At two ponds, one hose, and the helicopter pond, additional samples were collected from the middle (3) and bottom (3) of the pond. Edge and middle samples were taken c. 10 cm below the surface. Deeper samples were taken c. 70 cm above the bottom, to minimise the risk of substrate contamination.

To determine variation in the rate of breakdown within ponds, regular water samples were collected from the edge, centre surface and bottom of two ponds. Samples were taken at 3 h post application, 1 day after treatment and then at weekly intervals until an undetectable result (< 0.5µg/L) was recorded. To monitor variance between ponds, four other ponds were sampled from the edge only but at similar time intervals to monitor their rates of rotenone degradation.

LincLab Analytical Services in Christchurch measured rotenone concentrations in water samples. These were collected in 500 ml glass bottles filled to capacity and sealed with Teflon-line caps, and stabilised with 10 drops (10 ml) of phosphoric acid. Each sample was placed in black polythene bags, and cooled on ice within a chilli bin. At the time of collection, water temperature and conductivity were recorded from the pond edge, using a YSI Model 33 S-C-T meter and TScan conductivity meter, respectively. On arrival at the laboratory, samples were conditioned to room temperature and a 250 ml or 400 ml aliquot taken for analysis. The volume of aliquot was increased to 400 ml at a point in the sampling programme where it was necessary to improve sensitivity to a reportable limit of 0.5 ppb of rotenone. Aliquots were filtered via a 'Whatman®' 541 Paper (20–25 µm pore size) before extraction and concentration of rotenone onto an 'Alltech' HiFlow C18 (Cat# 215250, 500 mg) solid phase extraction cartridge. The cartridge was extracted with methanol to recover rotenone and made to a final volume of 5 ml. A portion of this extract was filtered through a 0.45 µm nylon membrane into a vial suitable for High Performance Liquid Chromatography (HPLC).

Rotenone concentration was determined in the extract by reverse phase HPLC with ultraviolet detection at 290 nm and calibration with external standards. The final reported figure was back calculated from this result to part per billion levels in the original water. Fortifying laboratory grade water with rotenone and analysing under the same conditions as samples, monitored recoveries. Recoveries were at a fortification level equivalent to 200 µg/L average 95.4% ± 6.4 with a 95% confidence (LincLab Analytical Services, pers. comm.).

2.2 Fish response

General behavioural observations were made at the first ponds treated to determine behaviour categories. A total of six behaviour categories were defined for each species and are described in Table 1. These behaviours are for fish at or near the surface. Water turbidity prevented observations at depth. At each pond for each species, the proportions of fish at the surface displaying each behavioural category were recorded continuously starting at the time of poison application. Proportions were estimated from the fish observed at the surface, and by back casting based upon the final numbers of dead individuals observed at the end of each operation. Time to first obvious surface reaction to the poison at each site, and the time when it was estimated that 99% of the population was dead or in a state of torpor, were also recorded. However, often the end point for tench and eels was not reached during daylight hours and therefore was not able to be recorded.

2.3 Fish revival

Revival trials were undertaken in four 60 L tubs, filled two-thirds (40 L) full with untreated water and aerated with a portable battery-operated aerator.

At the first ponds poisoned, shortfin eels were recovered as they came to the side of the treated pond, and placed in tubs containing different concentrations of methylene blue (30 mL, 60 mL, 90 mL or 300 mL of 5% methylene blue solution). In addition, poisoned juvenile (<100 mm) goldfish were also collected and held in tubs containing either 0 mL, 12 mL, 20 mL or 32 mL of 5% methylene blue solution.

TABLE 1. BEHAVIOURAL GROUPS ASSIGNED TO EACH SPECIES DETERMINED FROM AD LIBIDUM OBSERVATIONS IN FIRST PONDS POISONED.

SPECIES	A	B	C	D	E	F
Gambusia	Active surface swimming (ASS) and 'shimmering'	Slow surface swimming 'rippling'	Darting upright at water surface	Losing balance	Loss of balance: on side/ upside down	Torpor, floating on side/sunk
Rudd	Jumping out of water and (ASS)	Surface darting	Surface, stationary	Losing balance	Loss of balance: on side/ upside down	Torpor, floating on side/sunk
Tench	Surfacing and (ASS)	Slow swimming at surface	Vertical, stationary at surface	Losing balance	Loss of balance: on side/ upside down	Torpor, floating on side/sunk
Goldfish	Surfacing and (ASS)	Slow swimming at surface	Vertical, stationary at surface	Losing balance	Loss of balance: on side/ upside down	Torpor, floating on side/sunk
Koi	Surface lunging	Flipping out of water	Vertical, stationary at surface	Losing balance	As above, may roll over a few times	Torpor, floating on side/sunk
Perch	Surface lunging	Flipping out of water	Beaching	Losing balance	Loss of balance: on side/ upside down	Torpor, floating on side/sunk
Eels	Surfacing and (ASS)	Approaching shore	Beaching and/or retreating	Losing balance	Loss of balance: on side/ upside down	Torpor, floating on side/sunk

Fish were held until they had recovered fully or had died, and final numbers of each group were recorded.

At all sites general attempts were made to revive other non-target fish species including tench, rudd, shortfin and longfin eels, common bullies and goldfish. Fish were collected from the treated pond as soon as they became distressed and could be caught. They were then placed into aerated tubs containing enough methylene blue to colour the water light blue or into tubs of aerated untreated water. The time of capture was recorded and observations of fish behaviour noted. Any fish that survived for 24 h were transferred to a larger aerated 2000 L tank filled with fresh water and monitored for signs of relapse. Successful revival was defined as a return to normal buoyancy, orientation, movement and skin colour.

At one site a large number of poisoned eels were found the morning after application. These eels appeared to have surfaced overnight. All live eels were collected and placed in aerated water to which a large unmeasured dose of 5% methylene blue was added and their survival rate the following day (after 24 h of exposure) was also recorded. All fish still alive were then transferred to the large 2000 L tank.

3. RESULTS

A total of 17 ponds ranging in size from 0.05 ha to 4.4 ha (average 0.56 ha) were treated with rotenone in April 2001. Application methods are explained in detail in Shaw & Studhome (2001), but in brief at most ponds a concentrated rotenone solution was mixed up using a small quantity of Pulse™ surfactant to aid mixing. This solution was held in a recirculating 2000 L tank and then applied under pressure using a small portable fire pump and non-percolating fire hose. The solution was sprayed onto the surface and into the water to aid mixing, with a greater volume being applied to macrophyte beds around the pond margins. At one pond, rotenone was applied from a helicopter using a boom sprayer with the jets removed so the solution was discharged as large droplets. The deeper areas of the pond were given extra coverage.

At the first large pond poisoned, all fish were slow to react and it took almost 4 hours for the majority of fish to die. On the basis of the concentration applied (200 µg/L), the response rate was slower than previously observed (a small irrigation pond was poisoned for Gambusia in April 2000) and results reported in the literature (Willis & Ling 2000; Ling 2003). The rotenone used was up to 2.5 years old as it had been held by the Department for at least a year, and the supplier can also hold the powder for up to 18 months. In the absence of a testing facility (LincLab was not set up for measuring rotenone at this time) we assumed that the powder had degraded. Due to time and resource constraints, at the next ponds where old rotenone was used we doubled the application concentrations to compensate for the lower toxicity. The aim was to speed up the reaction time, and kill the fish more rapidly. The low rotenone concentrations subsequently measured at this pond (Table 2, H2) suggest some of the old stock was degraded.

TABLE 2. ROTENONE CONCENTRATION (µg/L) MEASURED FROM WATER SAMPLES COLLECTED FROM THE POND EDGE 3 HOURS POST POISON APPLICATION TO THE SURFACE WATER. Calculated concentration is the theoretical concentration based on estimated pond volume and quantity of rotenone applied. Concentration expected at the surface equals theoretical concentration based upon estimated volume of the top 1 m of the pond divided by the quantity of rotenone applied.

SITE	CALCULATED CONCENTRATION (µg/L)	CONCENTRATION EXPECTED IN SURFACE WATER (µg/L)	CONCENTRATION AT SURFACE 3 h AFTER APPLICATION (µg/L)
H2	264	264	68
H5	320	782	440 / 325 / 284
H6	322	726	385 /240
H7	732	733	474 / 302

Observations made at the ponds on the day of poisoning and in subsequent days indicates that all fish in the ponds were killed. All ponds were revisited repeatedly in the first 2 weeks following treatment, primarily to pick out dead fish but also to look for survivors. A young tench was observed in one pond (H7) 3 weeks after the operation but enquiries suggest that the tench had recently been released into the water body and was not a survivor of the poisoning. In spring 2001 and February 2002, the treated systems were resurveyed, and with the exception of this pond and a pond directly upstream on the same property (H8) no fish were found. Young tench were recorded in both H7 and H8, the latter again almost certainly representing a new release.

3.1 Rotenone monitoring

Concentrations of rotenone assessed 3 h post poisoning were highly variable for both the helicopter and hose application ponds (Table 3). Generally the highest concentrations were measured at the centre surface rather than the edge or centre bottom of either pond. At both sites the amount of rotenone applied was calculated to provide a concentration of 200 µg/L active rotenone. However, surface concentrations from helicopter spraying were higher than both the calculated concentration for the whole pond assuming full mixing ('calculated concentrations' hereafter), or the concentration expected if the rotenone was concentrated in the top 1 m of the pond ('surface concentrations' hereafter). The high, central surface helicopter concentrations probably reflect the higher volumes applied to areas over deeper water. Nevertheless, the helicopter spray application still achieved a relatively even spread of poison on the surface.

Hose application concentrations at all ponds, measured 3 h post application, were as variable (Table 2, Fig. 1), but were usually within 100 µg/L of the calculated concentration and considerably lower than the estimates for pond surface concentrations. Again some of the variance will reflect the uneven application with higher volumes sprayed onto areas of macrophyte or *in situ* fish cover.

TABLE 3. ROTENONE CONCENTRATION (µg/L) MEASURED FROM WATER SAMPLES COLLECTED FROM THE SURFACE EDGE, SURFACE MIDDLE AND BOTTOM OF THE POND 3 HOURS POST POISONING. Calculated concentration is the theoretical concentration based on estimated pond volume and quantity of rotenone applied. Concentration expected at the surface equals the theoretical concentration based on estimated volume of the top 1 m of the pond divided by the quantity of rotenone applied ([a] = broken samples).

SITE	TECHNIQUE	CALCULATED CONCENTRATION	EXPECTED SURFACE ($1m^2$)	ROTENONE CONCENTRATION µg/L EDGE	CENTRE SURFACE	CENTRE BOTTOM	DEPTH (m)
H14	Helicopter	208	679	814 593	744 605 700	227 106 [a]	2.5
H13	Hose	204	475	87 [a]	248 [a] [a]	82 51 [a]	1.5

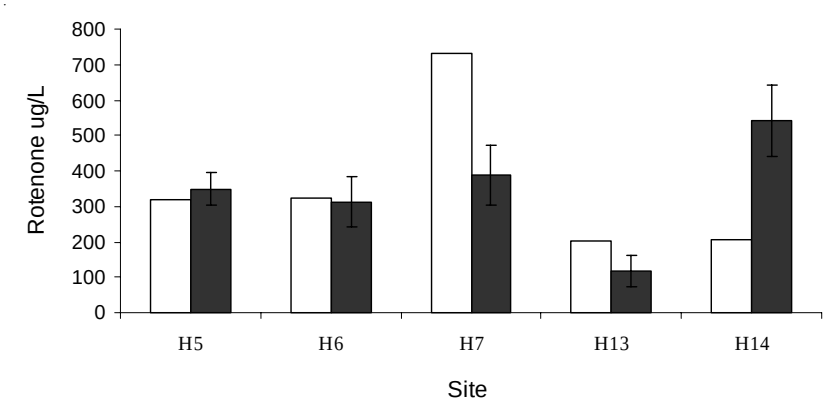


Figure 1. Average (+ 1 SE) rotenone concentration (black bar) at the surface measured from water samples collected from five ponds in Nelson treated in April 2000. Results are compared with the theoretical rotenone concentration (clear bar) calculated from estimated pond volume divided by quantity of rotenone applied. At pond H14 rotenone was applied by helicopter application.

The low concentrations of poison recorded in benthic samples (Table 3), is consistent with the powdered rotenone slowly settling out. This is supported by the rotenone degradation data (Fig. 2).

3.2 Rotenone degradation

At ponds H13 and H14, surface concentration at the edge and pond centre declined rapidly in the first 24 h, whereas benthic concentrations increased slightly, consistent with rotenone settling out (Fig. 2). Thereafter slow rates of decline were recorded throughout the ponds. In all ponds monitored (Figs 2 and 3) it took 15–50 days before concentrations had declined to detection limits (0.5–1 ppb). The rate of breakdown at H6 and H13 was considerably slower than at the other ponds, taking over 40 days for concentrations to drop to detection limits. Water temperatures recorded were variable at all ponds but were generally around 15°C , dropping at most sites through the course of monitoring (Figs 2 and 3).

3.3 Fish response

Reaction rates to rotenone varied between species although the order of response was consistent. *Gambusia* and rudd were the first fish observed, usually within 10–25 min of application (Figs 4 and 5), whereas eels, tench and goldfish were usually not observed until 40 min after treatment. Neither increased rotenone concentrations nor pond size appeared to shorten the initial

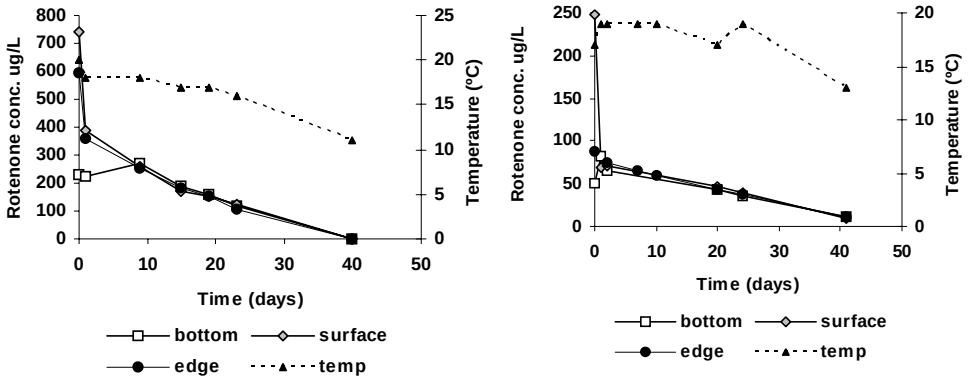


Figure 2. Changes in water temperature and persistence of rotenone at the edge, surface middle, and bottom of two ponds (H14 left, H13 right) following a single application of rotenone. Rotenone applied to pond surface from a helicopter (left graph) and fire hoses (right graph).

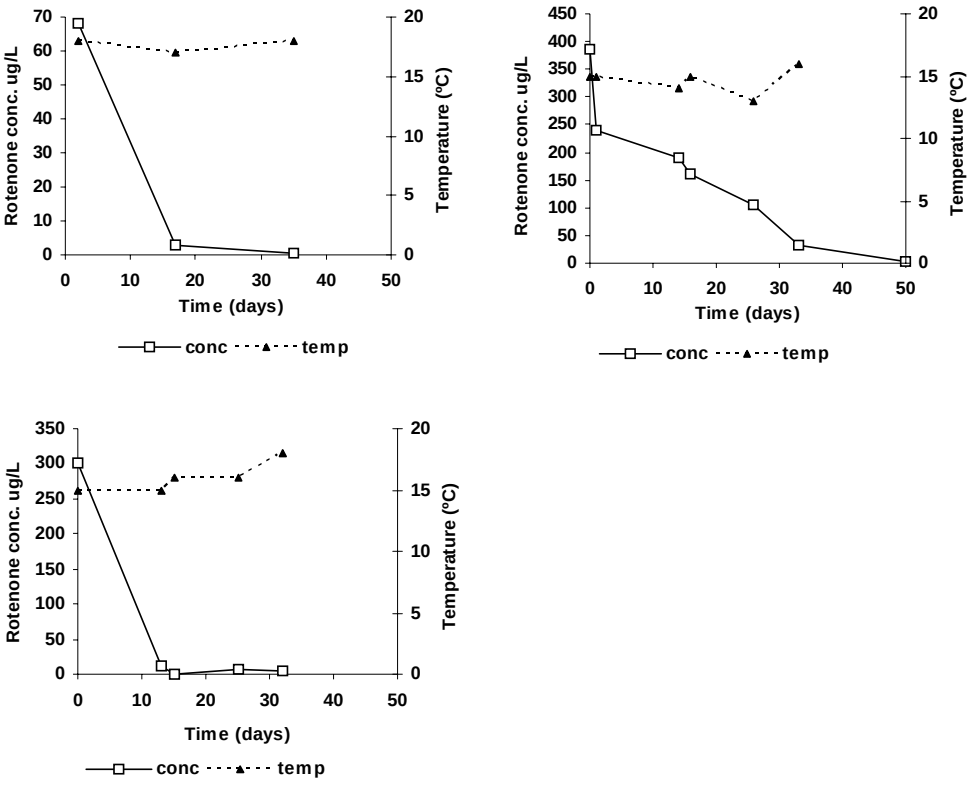
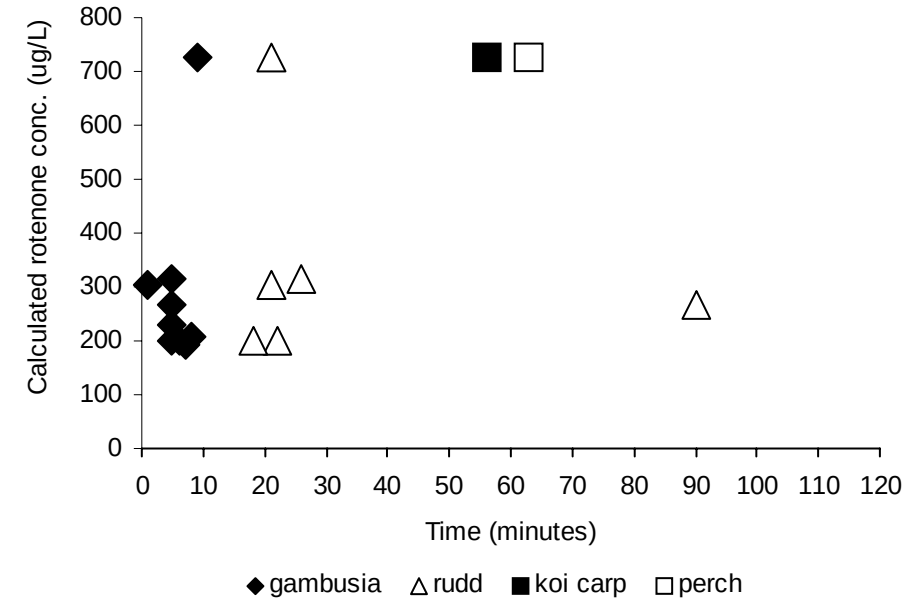


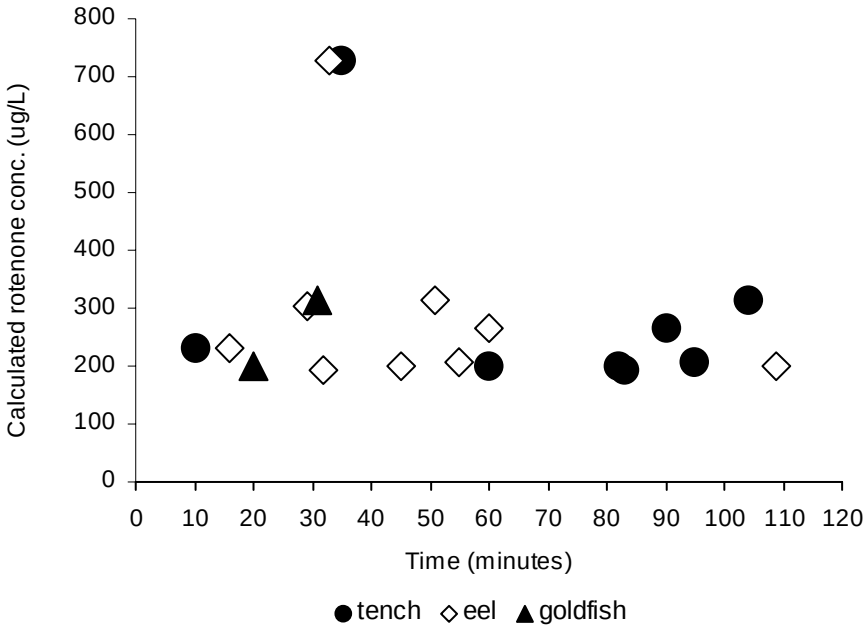
Figure 3. Changes in water temperature and persistence of rotenone at the edge of three ponds (H2 top left, H6 top right, H7 bottom) following a single application of rotenone applied with fire hoses.

reaction time of any species (Figs 4 and 5), except in the smallest ponds where fish were observed soon after poison application. For example in pond H16, a small 1700 m^3 pond, tench and eels were observed only 16 min after rotenone application (calculated rotenone concentration 308 µg/L). Koi and red finned perch were observed at only one pond (H7) and these species surfaced after 56 min and 63 min, respectively.

Six general response categories described below were assigned for all species as they tended to react to rotenone in a similar manner with minor variations (Table 1). The initial reaction for most species was frantic swimming at the water surface (*active surface swimming*). As the length of exposure time increased, activity at the surface lessened. Fish usually remained at the surface, upright and still swimming (*slow surface swimming*) although at regular intervals individuals frantically darted across the surface and then resumed swimming upright very slowly (*darting*). They would then begin to lose

Figure 4. Time until first observed reaction to an application of rotenone and the theoretical rotenone concentration present in the top metre of the pond (i.e. quantity of rotenone applied divided by area present in the top 1 m of the pond).

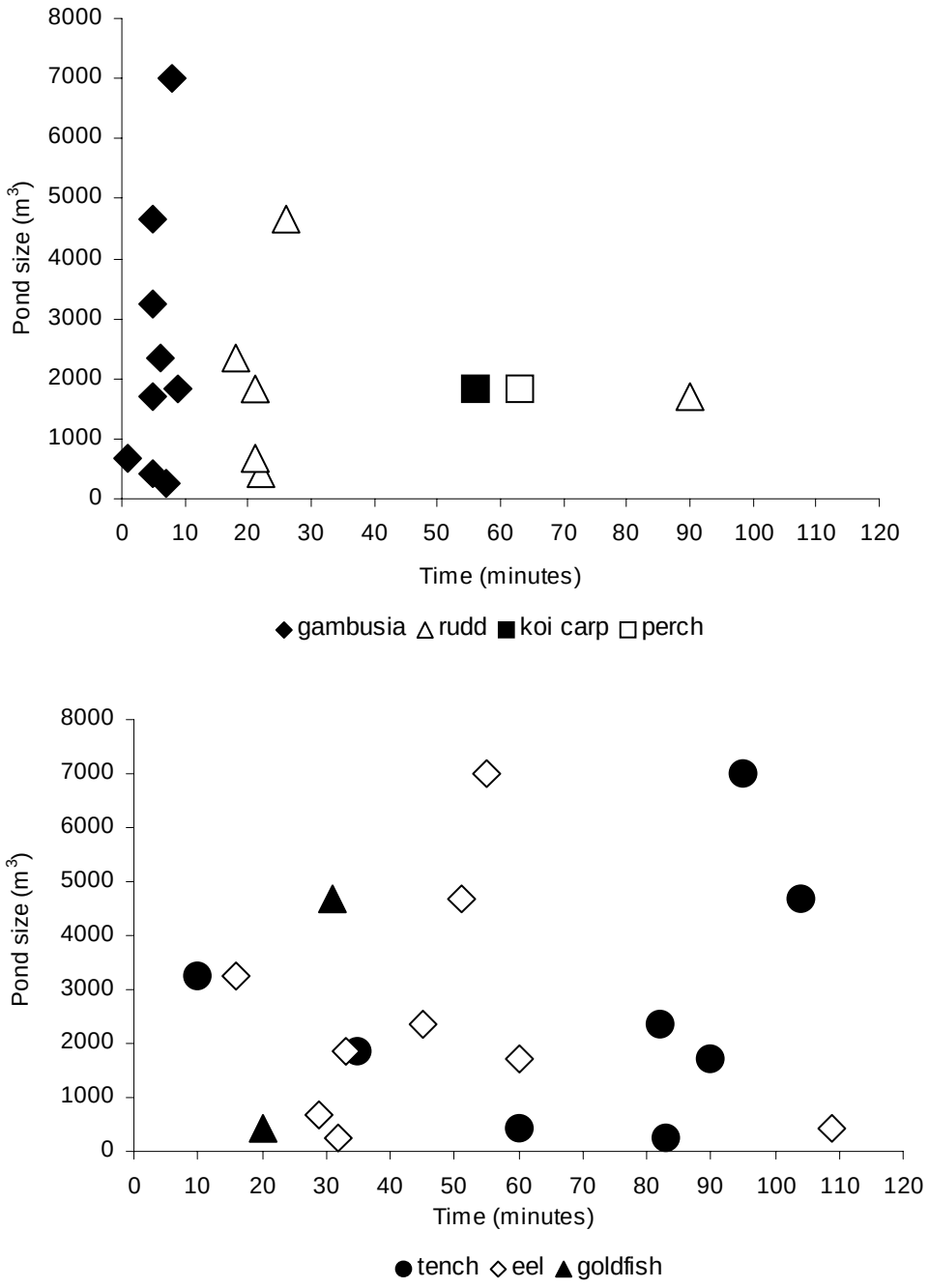




equilibrium or orientation and often whilst still swimming they would roll over onto one side, right themselves and roll over again (*losing buoyancy*). Eventually the fish were no longer able to right themselves and hung on their sides or sometimes upside down at the water surface, often twitching (*belly up*). Finally the fish became completely immobile, usually floating on their sides or belly up (*torpor or death*).

Gambusia was usually the first species observed to react to poisoning. Within about 10 min (Figs 4–6) the surface of the pond began to ripple as the *Gambusia* came to the surface. *Gambusia* normally respond to hypoxia by skimming oxygenated surface water across their gills (Willis & Ling 2000), which is possibly what was observed. The fish rapidly progressed to slow

Figure 5. Time until first observed reaction to an application of rotenone and pond size (m^3).



surface swimming then into the darting behaviour characterised by short bursts, with smaller fish beginning to loose orientation after as little as 15 min (Fig. 6). The small fish succumbed rapidly within about 20–25 min and typically sank once torpor had set in, whereas large females often maintained orientation and movement for 20–30 min, and in some instances they were still alive up to 3 h post poisoning (Fig. 7).

Rudd appeared at the pond surface typically 5–10 min after *Gambusia* had begun to surface skim (Fig. 6). Their reaction to rotenone was usually violent, with fast active surface swimming that progressed into frantic jumping out of the water and thrashing around at the surface. They rapidly lost buoyancy control and orientation and hung tail down at the surface, but rapid darting and additional jumping behaviour often punctuated this. Total loss of buoyancy

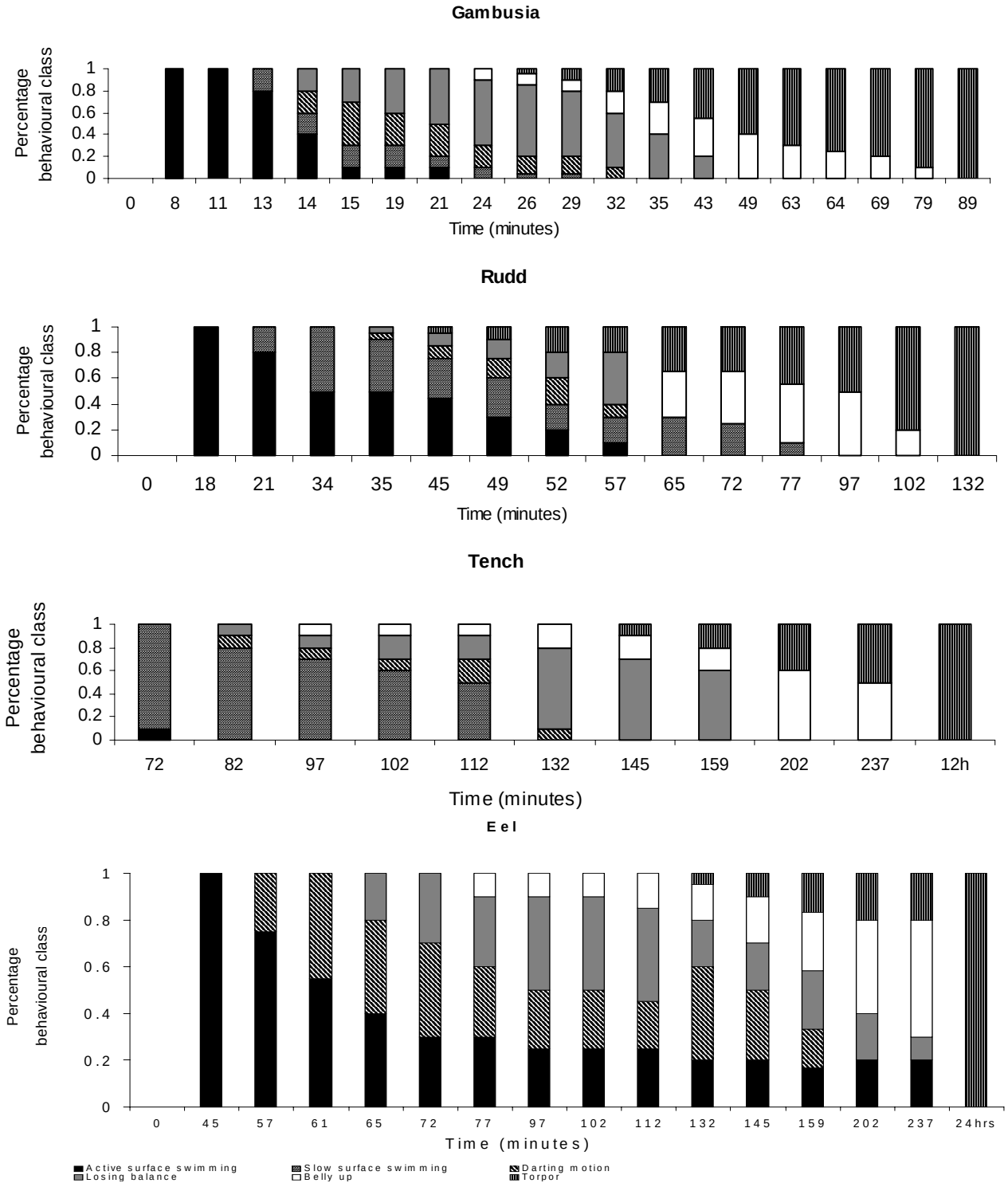


Figure 6. Representative examples of temporal changes in expression of behaviour classes following exposure to rotenone (0 = time which rotenone was applied). Note different horizontal scales.

control rapidly followed and the fish lay on their side or belly up at the surface as torpor set in. The last rudd were usually dead by the same time or just before the last large female *Gambusia* had succumbed (Fig. 7).

Tench, shortfin eels and goldfish were slow to react. Juvenile tench and shortfin eels (including elvers) were the first of these fish to appear, and were often observed swimming frantically at the water surface away from

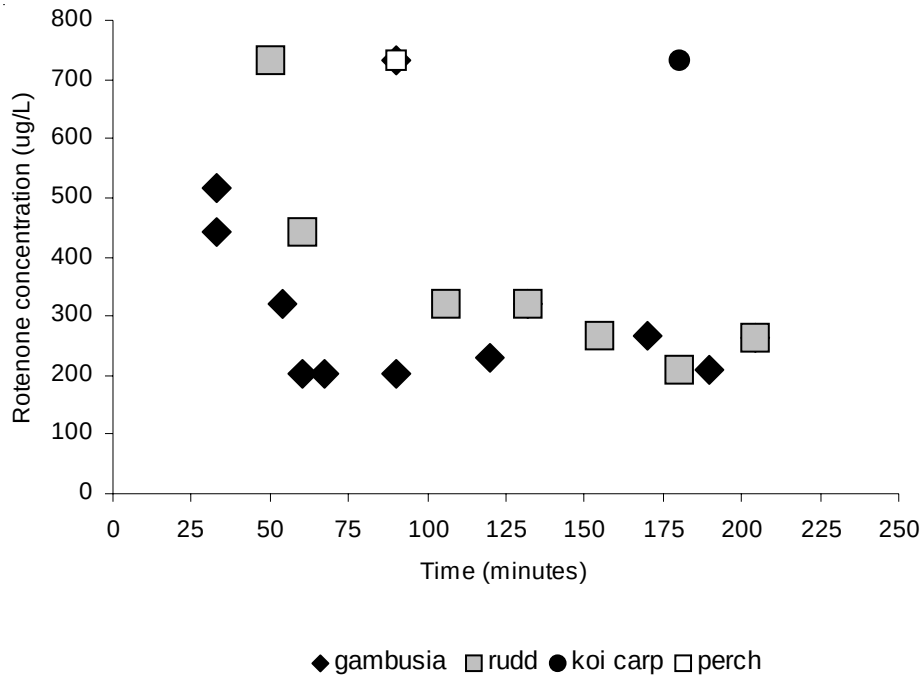


Figure 7. Time taken until 99% of population was estimated to have died and the theoretical rotenone concentration present in the pond (i.e. quantity of rotenone applied divided by whole pond area).

macrophyte cover. They generally lost equilibrium within minutes of being first observed, often before large adult fish had appeared at the surface (Fig. 6). The first large eels were usually observed within 30–40 min of application, whereas in the absence of juvenile fish it was often over an hour before the first tench appeared (Figs 4 and 5).

Mature tench, rudd, goldfish, koi and perch followed similar behaviour patterns to *Gambusia* but were also observed porpoising and lunging through the surface water and sometimes jumping clear of the water. These behaviours were repeated at intervals. As the effects of rotenone set in, the larger fish slowed down and tench and koi began to roll over repeatedly at the water surface as they were swimming. Large adult tench were often the last fish observed doing slow barrel rolls at the surface 2–3 h after poisoning.

Eels reacted by swimming across the water surface, and as they became more stressed they would swim frantically toward the edge of the pond and attempt to leave the water. Where successful, they lay motionless on the banks, gulping air. Otherwise they often accumulated at the pond edges, lying on their sides in an apparent state of torpor. However, if disturbed they were capable of swimming away in short rapid bursts that made capture difficult. Torpid eels began to accumulate after about an hour, but active fish continued to appear over the next 2 h (Fig. 6) and live adults were often found the following morning.

The rate at which an estimated 99% of the *Gambusia* population had succumbed generally decreased with increased rotenone concentration (Fig. 7). The shortest time recorded was at H4 (calculated concentration 403 µg/L), where surface 'shimmering' was observed after 6 min, and within 33 min of application the population of *Gambusia* in the pond had either sunk or were in a state of torpor, presumed dead. The longest time recorded was 204 min at H2 (calculated concentration 264 µg/L, actual measured concentration 68 µg/L).

Rudd displayed similar levels of sensitivity, taking between 50 min and 204 min post application to reach 99% mortality, with the shortest times recorded in ponds with highest concentration of rotenone (Fig. 7). Although rudd were usually seen at the surface after the *Gambusia* were first seen reacting, at 71% of the ponds where both species were found, all the rudd were dead before the last *Gambusia*.

Eels, goldfish and large tench typically survived post treatment for longer than observations were made. Although the majority of fish had entered a state of torpor, small numbers of fish were still mobile many hours after application. The time taken for 99% of these species to die could therefore not be accurately recorded. For instance large mature tench remained alive until observations were terminated 7 h post poison application at one pond, and none were observed alive the following day. At one pond, live eels were found 24 h after application and at H2 two large goldfish were still alive the following morning.

3.4 Fish revival

Attempts were made to revive shortfin eels, common bully, tench, goldfish and rudd. Fish were collected as soon as possible after poisoning and placed in aerated tubs of clean water, with or without methyl blue added.

Survival of all size classes of goldfish and shortfin eels were high where these species were collected within the first 1–2 h of treatment. All goldfish usually recovered rapidly even in untreated water and there was no indication that methylene blue (concentrations 0.3 mL/L, 0.5 mL/L, 0.8 mL/L) aided recovery. Shortfin eels were slow to recover. All fish usually entered a state of torpor soon after being placed into aerated tubs. They lay on their sides, rigid, and would not respond to touch. However, skin pigmentation and eye colour retained a healthy look. Eels remained in a state of torpor for about 48 h before regaining orientation and active swimming. Provided the water was well aerated, any eels still alive after 24 h usually recovered. Generally, over 90% of the eels collected within 1–2 h of poisoning recovered, and just over 60% of the eels collected 24 h after poisoning were also successfully revived.

There was no evidence that increased concentrations of 5% methylene blue solution increased the rate of survival of shortfin eels or goldfish.

Large tench after prolonged (1–2 h) exposure were successfully revived in aerated water coloured with methylene blue (6 out of 9 revived) whereas no small tench were revived (n = 9). However, these small fish were held initially with eels and larger tench and were heavily slimed which may have impeded recovery. All attempts to revive rudd and *Gambusia* were unsuccessful.

The revival success of common bullies was varied. If held in high densities and vigorous aeration, survival was poor, whereas small numbers of fish were revived when held at low densities in low-turbulence, well-aerated water (66% recovery).

4. DISCUSSION

Gambusia, koi, rudd, perch, tench and shortfin eels appear to have been eradicated from 17 ponds in the Nelson region by a single application of rotenone. Hose and helicopter boom-spraying were both effective at applying rotenone across the ponds at a high enough concentrations to kill all fish.

Rotenone concentrations measured on the day of application varied across and between ponds and application technique. Greater amounts of rotenone were applied to pond edges and macrophyte beds, yet the concentrations of rotenone at the edge were lower than expected. The water jet from the hoses stirred up sediment at shallow margins, and it is possible that as the sediment settled out it bound up, or carried, the rotenone to the pond bottom as it is readily absorbed by sediment (Dawson et al. 1991).

A relatively even spread of rotenone was achieved using the helicopter sprayer and the concentrations measured were within expected levels given that it was applied to the pond surface. Monitoring results at surface, centre middle and bottom indicate the powdered rotenone was slowly settling out over 24 h, consistent with the low solubility of rotenone powder.

Rotenone persisted in the ponds for longer than expected. The degradation of rotenone in aquatic environments is influenced by many environmental factors that vary seasonally (Gilderhus et al. 1988), including, temperature, water volume, acidity/alkalinity, surface area, substrate, sunlight, turbidity and dissolved oxygen (Bettoli & Maceina 1996). Phytoplankton, zooplankton and bacteria are also likely to influence the rate at which rotenone disappears from water and are likely to be most active in warm water (Gilderhus et al. 1988). The influence of temperature has been well documented (Post 1958; Engstrom-Heg & Colesante 1979; Gilderhus et al. 1986) with rapid degradation at high temperatures (>20°C) and very slow breakdown at temperatures below 10°C.

The slower rates at H6 and H13 may reflect the low light penetration at these ponds as a result of high turbidity and tannin staining, respectively. Gilderhus et al. (1988) indicated that temperature and light penetration strongly influence the rate of breakdown and our results are consistent with this. At lower sunlight levels, rotenone will remain toxic for long periods, weeks or even months (Sanger & Koehn 1997). However, Finlayson (2002) felt that the persistence of rotenone for up to 4 weeks was probably caused by the high application rates and the acidic nature of the water. Rotenone decays under first order kinetics and, in waters with neutral pH or above, the half-life of rotenone would vary from 0.6 days to 7.7 days, depending on water temperature and depth (Finlayson et al. 2001). Hence if the influence of pH is discounted, the shallow depths and temperature range (16–20°C) of the waters treated should have yielded a half-life of 1–2 days with rotenone degrading from 400 µg/L to <2 µg/L in 8–16 days (Finlayson 2002).

Some of the loss of rotenone may have reflected the continued settlement of undissolved rotenone, reflecting its low level of solubility (200 µg/L at 20°C, USPEA 1988). Thus the high levels detected probably represent a large amount of undissolved rotenone suspended in the water column. Rotenone that settles out would be expected to continue to decay in the sediment as shown by Dawson et al. (1991).

Rudd and *Gambusia* were the first species seen to react to rotenone. They are pelagic species usually observed at or near the surface, with *Gambusia* favouring shallow margins where rotenone spray was concentrated. Species such as tench and eels were slower to react, probably reflecting their benthic behaviour and the time taken for rotenone to settle to the bottom of the ponds, as well as their higher tolerance to rotenone and low oxygen conditions. In shallow ponds, settlement was faster and these species were observed at the surface within a shorter time period. The amount of rotenone applied to each pond varied with estimated volume, and as a result fish were exposed to different strengths of rotenone. As would be expected, the stronger concentrations generally resulted in a more vigorous and rapid the response.

Rotenone causes oxygen deficiency at the cellular level by blocking aerobic metabolism (Fajt & Grizzle 1993), causing many fish to gasp at the water surface (Willis & Ling 2000). Tench, goldfish, koi and perch appeared to minimise water flow over their gills by reducing opercular movements and hung almost motionless at the water surface gulping air. A similar response was noted for large rudd at ponds where low concentrations of rotenone were applied. *Gambusia* were observed skimming surface water across their gills as documented by Willis & Ling (2000). Shortfin eels were observed surfacing and swimming rapidly toward the shore where they would lie still, gulping air. All fish species followed the same generalised pattern of rapid movement followed by slower darting motion, loss of equilibrium and finally inertia and death.

Rudd appeared to be more susceptible to rotenone than *Gambusia*. Typically a few large *Gambusia* would outlast the final rudd even though the former were the first to react. Goldfish, tench and shortfin eels were the most resistant species. Koi and perch were observed at only one pond. Koi survived prolonged exposure under extremely stressful conditions whereas the single perch observed died shortly after the first sighting. These results are consistent with known rotenone tolerance for these or related species (Hamilton 1941; Fabacher & Chambers 1972; Meadows 1973; Marking & Bills 1976; Fajt & Grizzle 1993; Waller et al.1993; Willis & Ling 2000). As expected, the time taken to reach 99% mortality for each population generally decreased with an increase in rotenone concentration, and the largest fish were the last to succumb.

The different tolerances to rotenone between species, particularly the high sensitivity of rudd versus high tolerance of eels, may allow more species-specific poisoning. Using either low dose rates or a neutralising agent like pottassium permanganate could enable rudd to be selectively removed from sites, with minimal impacts on the resident eel populations. Selective poisoning has commonly been used in the United States to manage coarse fish populations (Ling 2003), often exploiting different thermal layers in lakes to kill coarse fish while minimising impacts to salmonids that are very sensitive. The tolerance of New Zealand native fish and the optimal rotenone concentrations to kill rudd need to be tested to enable selective removals to be undertaken.

Early studies found that fish exposed to rotenone did not survive once they had lost equilibrium (Leonard 1939; Bassett 1956). However, we were able to successfully revive large tench and shortfin eels at least in the early stages of equilibrium loss. However, we were not able to revive *Gambusia* or rudd.

The length of exposure before capture negatively affected revival success. The literature on toxicity of rotenone to fish suggests that concentrations used in fishery management are generally higher than those known to be lethal in laboratory tests, (Schnick 1974; Meyer 1966). During this project, eels and goldfish were found to survive extended periods in treated ponds, with the former surviving up to 24 h post treatment where low concentrations were used and where muddy substrates were present. Revival of some of these eels was successful.

Treatment with methylene blue has been found to reduce the respiratory inhibition caused by rotenone (Lindahl & Oberg 1961). In laboratory tests, inhibition of the O_2 uptake in gill filaments, as well as in mitochondria, was reversed by the addition of methylene blue. We could find no evidence that the addition of methylene blue increased the revival rate of the eels and goldfish in the tubs, as a high percentage of the fish in untreated water also made a full recovery. However, eels and goldfish in the container with the methylene blue appeared to recover much faster than fish in untreated water and we would still recommend its use and further more comprehensive trials.

5. CONCLUSIONS AND RECOMMENDATIONS

- Both application techniques used were effective at spreading rotenone relatively evenly despite problems calculating pond volumes.
- Rotenone was found to degrade slower than expected, probably due to low pH, and took 15–50 days to dissipate in farm ponds.
- There was considerable variance in the rate of reaction between fish species, which was found to emulate previous studies on fish sensitivities and reactions to rotenone. Rudd and *Gambusia* were the most sensitive; tench, goldfish and eels the least. Rates may also reflect the relative habitats of each species and their activity patterns.
- The different rates of reaction indicate potential for selective removal of sensitive species. This would require a refinement in application techniques.
- Revival methods, using oxygenated water, were successful for shortfin eels and common bully. Goldfish and large tench were also successfully revived.
- Recovery rates appear to be improved by the addition of methylene blue, but this was not supported by revival data.
- Rotenone tolerance of other native fish species needs to be tested as well as their ease of revival following poisoning.

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