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Conservation of the endangered red-finned blue-eye, *Scaturiginichthys vermeilipinnis*, and control of alien eastern gambusia, *Gambusia holbrooki*, in a spring wetland complex

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Abstract. The red-finned blue-eye is the only pseudomugilid fish known from inland Australia and it is found only within an isolated cluster of Great Artesian Basin springs on Edgbaston Reserve in central-western Queensland. Surveys conducted in early 2009 revealed that red-finned blue-eye was present in four individual springs and that invasion of the spring complex by alien eastern gambusia was the most likely factor contributing to local extirpations. A three-year project commenced in the same year, with the twin aims of investigating methods for removing gambusia from springs and relocating small populations of red-finned blue-eye to fish-free springs. Gambusia removal with rotenone has been successful in a trial spring at Edgbaston and aquatic invertebrates have not been adversely affected. From a total of seven relocation events conducted in the same period, red-finned blue-eye populations have persisted in three. The results indicate that gambusia removal and red-finned blue-eye relocation are both suitable methods for red-finned blue-eye conservation, and as the fish is both endangered and declining, these methods and other strategies such as captive breeding should be implemented to prevent species extinction.

Additional keywords: endangered species recovery, Great Artesian Basin, invasive species control, Lake Eyre Basin, rotenone.

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Introduction

In freshwater systems, invasion by alien fish has a dramatic impact on native fish decline (Minckley and Deacon 1991). The impacts associated with alien species are magnified in isolated aquatic ecosystems, particularly in water-remote arid areas (Box *et al.* 2008), and these impacts are most damaging in areas where there is high endemicity of resident biota (Fensham *et al.* 2011). In isolated arid-zone aquatic ecosystems such as the Great Artesian Basin spring complexes in inland Australia, the potential for species decline and loss is therefore extremely high. Captive breeding and/or relocation of fish are techniques frequently employed for conserving threatened species (Philippart 1995) because the control and removal of alien species is often difficult or impossible (Scoppettone *et al.* 2005).

The red-finned blue-eye, *Scaturiginichthys vermeilipinnis* (Fig. 1), was discovered in 1990 by Peter Unmack in spring-fed waters at Edgbaston station, north-east of the town of Aramac in central-western Queensland (Fig. 2). Edgbaston is located in the semiarid Thomson River catchment which is part of the Lake Eyre Basin, and became a reserve in 2008 following acquisition by the not-for-profit conservation organisation Bush Heritage Australia. The red-finned blue-eye is the only pseudomugilid fish known from inland Australia, with other blue-eyes generally found in coastal draining rivers of northern and eastern Australia and New Guinea (Allen *et al.* 2002, 2008). The red-finned blue-eye reaches a maximum length of 3 cm and has been recorded only from the spring complex at Edgbaston. The species is listed as *Endangered* under both national and state legislation (NCA 1992; EPBC 1999), as *Critically Endangered* by the IUCN (2012), and has recently been included in a book published by the IUCN highlighting the plight of the 100 most endangered species worldwide (Kerezsy 2012*a*).

The Great Artesian Basin springs at Edgbaston are isolated aquatic ‘islands’ within a semiarid landscape. Currently, there are up to 100 springs, soaks or damp areas present at Edgbaston, and the amount of water within each spring ranges from moist areas or small puddles to areas up to 30 m wide. Despite variation in the extent of wetlands depending on the moisture status of the substrate, water depth within the springs rarely exceeds 5 cm because of the flat landscape. Over long time-frames, groundwater discharge to the springs may have been diminished by water extraction through artesian bores (Fairfax

Fig. 1. A mature, male red-finned blue-eye sampled from Spring NW30 on Edgbaston Reserve in March 2009. Photo: Adam Kerezsy.

et al. 2007). The springs contain slightly saline water (generally up to 1 mS cm^{-1}) that emerges, devoid of dissolved oxygen, at a constant temperature of ~24°C from the spring vents. However, when the water is distributed within the springs, it becomes oxygenated and the temperature fluctuates in relation to season and time of day (from close to freezing in winter to close to 40°C in summer).

Discharge springs such as the complex at Edgbaston have been identified as priority areas for conservation in the central Australian arid and semiarid zones, using the criterion of endemicity (Fensham *et al.* 2011), and the aquatic biota at Edgbaston is the most species-rich of any spring complex in Australia as a result of the diversity of endemic fishes, plants and invertebrates. In addition to red-finned blue-eye, the Edgbaston goby, *Chlamydogobius squamigenus*, occurs in at least 10 local

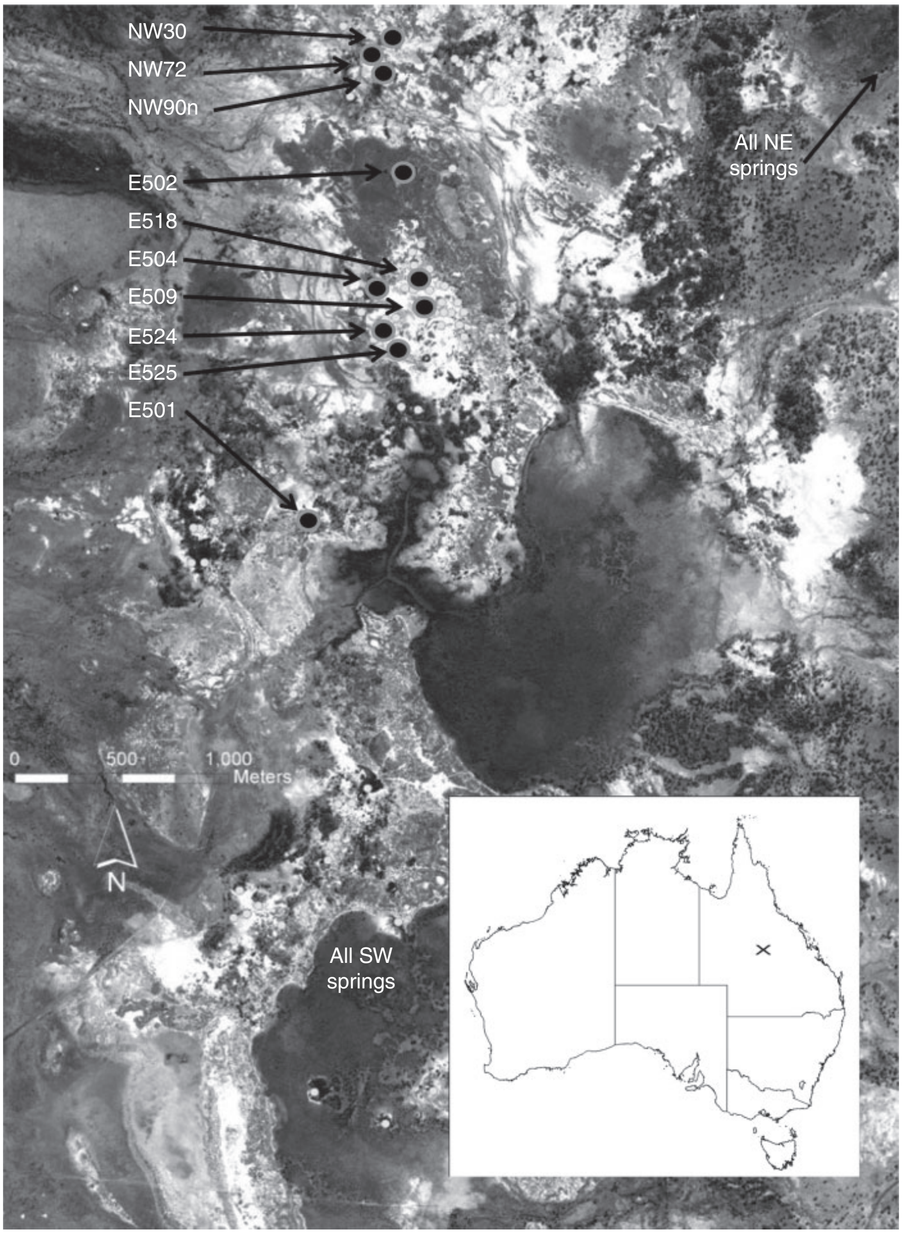

Fig. 2. The location of some of the individual springs and relative location of spring groups at Edgbaston, and (inset) the location of the property in central-western Queensland.

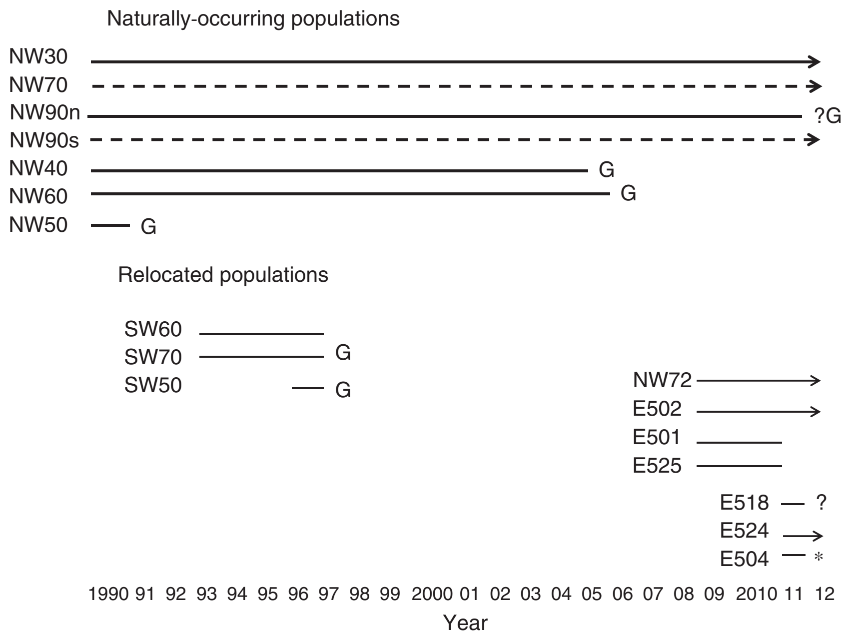

Fig. 3. The presence/absence of red-finned blue-eye at individual springs since the species was discovered. Solid lines = continual presence on all sampling occasions, dotted lines = intermittent presence, arrows = present during sampling in 2012, question marks = absence during sampling in 2012 and possible local extirpation, asterisk = relocation attempt aborted and fish moved back to spring of origin. The letter 'G' denotes colonisation of the spring by gambusia. All records before 2009 were summarised from Fairfax *et al.* (2007).

springs and a large number of endemic aquatic snail species from the hydrobiid, planorbid and bithyniid families are present (Ponder and Clark 1990). Both the ecological community and extant individual species at Edgbaston have been listed under endangered species legislation and are the subject of recovery plans (NCA 1992; EPBC 1999; Fensham *et al.* 2007, 2008).

Temporary floodwaters provide colonisation opportunities between the isolated springs for all aquatic biota at Edgbaston, and this includes the alien fish eastern gambusia, *Gambusia holbrooki*. Although the origin of eastern gambusia (henceforth 'gambusia') at Edgbaston is unknown, red-finned blue-eye populations declined from seven to four springs between 1990 and 2007 (Fig. 3), with colonisation of the springs by gambusia the most likely causal factor (Fairfax *et al.* 2007). Gambusia has been demonstrated to have deleterious effects on native Australian freshwater fish (Ivantsoff and Aarn 1999) and specifically on a related member of the pseudomugilid family, *Pseudomugil signifer* (Howe *et al.* 1997). Although the exact mechanism(s) by which gambusia affects red-finned blue-eye is unknown, the recorded patterns of local extirpation (in both Fairfax *et al.* 2007, and also more recently by the authors, Fig. 3) have indicated that these events are always accompanied by gambusia infestation. The urgency of the ecological situation at Edgbaston is exemplified by the most recent invasion of Spring NW90n (Figs 2, 3); before mid-2011, the spring contained one of the last naturally occurring populations of red-finned blue-eye; however, by the following summer gambusia was the only fish present.

The threats to red-finned blue-eye – a naturally restricted distribution combined with the imposition of an invasive species – were recognised shortly after its discovery. Prior to being listed as an endangered species, it was raised in captivity and attempts were made to establish translocated populations at Edgbaston (Fairfax *et al.* 2007). However, former keepers and collectors of the species confirm that no captive populations have endured or currently exist (Syd Adams, Gunther Schmida, Leo O'Reilly, Peter Unmack, Steve Brooks, pers. comm., 2011 and 2012). Additonally, all translocations undertaken in the early 1990s (Wager 1994; Fig. 3) have failed, most probably as a result of colonisation by gambusia and/or drying of receiver springs (A. Kerezsy, pers. obs., 2009 to present).

In recognition of the red-finned blue-eye extinction threat, Bush Heritage Australia began a project in 2009, with the aims of investigating methods of controlling gambusia and relocating populations of the endangered species to springs where no fish (of any species) were present. Results from the initial phase of this project are presented, and the future of the project is discussed with reference to factors that are likely to have an impact on its implementation and success.

Materials and methods

Gambusia control

Gambusia control using physical removal methods (netting) was trialed in the first instance at Edgbaston, in order to minimise the potential harm to non-target organisms in the endangered ecological community. However, this method was deemed unsuitable for all but the smallest springs because of the difficulty of netting gambusia to depletion in such complex habitat. Removing small numbers of gambusia (<500) from a spring with a small surface area (<3 m^2) took up to 6 months and included multiple visits to the spring at different times of the day (Kerezsy 2009).

Gambusia control using the piscicide rotenone commenced in 2009. This chemical was chosen because of its effectiveness in enclosed waters (Lennon *et al.* 1970; Rayner and Creese 2006) and its availability as a registered control technique for invasive fish in Australia. Rotenone has been used only in springs where extensive surveying has indicated that no threatened fish species (red-finned blue-eye or Edgbaston goby) are present. However, given the wide distribution of threatened non-target organisms throughout the Edgbaston spring complex, the focus of the rotenone work was two-fold, namely, to determine the effectiveness of the chemical for controlling gambusia and to determine any deleterious effects on non-target organisms.

Rotenone dosage rate

Rotenone occurs naturally in plants of the *Derris* genus (family Fabaceae) in the south-western Pacific and South-east Asia. Crushed or powdered roots of suitable plants have been used in traditional fisheries (Bearez 1998; Ling 2003) and this led to historical usage of the plant in fisheries management at dosage rates of up to 2 ppm (Leonard 1939; Brown and Ball 1943). A simple experiment was conducted in mid-2009 to determine the lowest dosage rate of rotenone required to kill gambusia, so as to minimise possible harm to non-target species (aquatic invertebrates). Powdered derris root (8% active ingredient) was obtained from the Queensland Department of Primary Industries.

Six glass aquariums (600 × 300 × 300 mm) were filled with 50 L of harvested rainwater and 180 gambusia individuals were collected from Humpybong Creek (Redcliffe, Brisbane) using six un-baited bait traps (400 × 300 × 300 mm with a 40-mm-diameter entry hole, set for 15 min). The fish ranged in size from 15-mm to 38-mm standard length and comprised both males and females. Fish were transported back to the aquaria facility in oxygenated buckets. Thirty gambusia individuals were released into each aquarium and allowed to acclimatise to captive conditions for 24 h. The water in the aquariums was not aerated or filtered, primarily because this was considered to best replicate conditions at Edgbaston. The experiment was conducted at ambient water temperatures ranging from 17°C in the mornings and evenings to 20°C during the middle of the day. Rotenone was mixed at 0.16, 0.32, 0.48, 0.64, 1.28 and 2.56 ppm by combining 0.1, 0.2, 0.3, 0.4, 0.8 and 1.6 g, respectively, of powdered derris root with a small quantity of water in separate watertight jars. Each jar was shaken for at least 60 s, so as to dissolve the powdered derris root, and the dissolved derris-root solution was then added to five of the six aquariums. The sixth aquarium was a control and no derris root was added. Each aquarium was observed at 30, 60, 120, 180, 240 and 300 min after derris root/rotenone addition and dead gambusia individuals were counted and recorded. Inspections were repeated 24 and 48 h after the rotenone was first introduced. At the conclusion of the experiment, all aquariums were drained and remaining gambusia individuals were euthanased with a dilute Aqui-S solution sourced from Queensland Department of Primary Industries.

The effect of rotenone on aquatic invertebrates – tank trials at Edgbaston

Tank trials were carried out at Edgbaston to determine whether non-target species are adversely affected by rotenone treatment. Three glass aquariums (600 × 300 × 300 mm) were transported to Edgbaston and located in a shaded position. Each aquarium was filled with water collected using plastic buckets from Spring E509, which is located in the central section of the spring complex (Fig. 2). The experiment was conducted at ambient water temperatures, commencing at 23.5°C and concluding at 25°C.

Invertebrates were collected from Spring E509 for 2 h on the evening before the commencement of the experiment by using dip nets and they were identified using a relevant field guide (Ponder *et al.* 2010). Aquatic invertebrates were distributed between the three aquariums. Fifty snails comprising *Gyraulus*, *Gabbia* and *Jardinella* sp. and ~100 shrimps, *Caridina thermophila*, were counted into each aquarium. Aquatic invertebrates sampled in small numbers, such as beetles, mites and leeches were also distributed among the aquariums in equal numbers where possible. Gambusia individuals were collected from E509 with a seine net (5 m × 1 m) with 2-mm mesh. The fish ranged in size from 12-mm to 45-mm standard length and comprised both males and females. At the commencement of the experiment (0800 hours, 23 October 2009), 50 gambusia individuals were released into each aquarium and allowed to acclimatise to captive conditions for 2 h.

The water in the aquariums was not aerated or filtered for the duration of the experiment. At 1000 hours on 23 October 2009, each aquarium was treated with rotenone at a dosage rate of 0.32 ppm, as per the methods described above. The aquariums were inspected at 1100 hours, 1200 hours and 1300 hours and mortalities of gambusia and invertebrates were counted and recorded.

Dead gambusia and shrimps were removed from the aquariums at 1300 hours and living snails, beetles, shrimps and mites were returned to Spring E509.

Rotenone application in a spring

Rotenone treatment commenced in Spring E509 in late February 2011. The treated area of the spring was ~20 m^2, with an average depth of 5 cm. The powdered derris root was mixed with water and a marker dye such that the rotenone concentration was 0.32 ppm and was applied using a backpack spray unit. The powdered rotenone clogged the spray nozzle within a few minutes, so the mixture was delivered using the spray lance without the nozzle head.

Thick vegetation throughout the spring prevented effective coverage using the rotenone mixture, so over the next month, the spring vegetation was sequentially cut back with a brushcutter. A dilute glyphosate mixture (Roundup Plus, Monsanto, at 15 mL per 15 L) was then applied to remove dense vegetation from the deeper areas of the spring. Rotenone was re-applied in early May, on two consecutive mornings. Trials in May used a combination of different spray units, including backpack, quad-bike mounted and a vehicle-mounted unit.

Rotenone was re-applied throughout the spring on two consecutive mornings in August 2011 and once in September 2011. Because all treated areas of Spring E509 were within 6 m of the main spring vents, the temperature of the spring water remained close (±2°C) to 24°C throughout each treatment; however, water temperatures reached 28°C during the September treatment. Monitoring of Spring E509 for the presence of gambusia (visual inspection of the spring for 30-min periods

Table 1. Categories used for sorting invertebrates and fish from Spring E509 during recent monitoring work at Edgbaston, and the total numbers of each category sampled before and after rotenone application

Species or category	Comments	Totals before rotenone (November 2010 and February 2011 combined)	Totals after rotenone (September 2011 and February 2012 combined)
Jardinella edgbastonensis, *J. corrugata* and *Edgbastonia allanwillsi*	Short-spired hydrobiid snails that are difficult to tell apart	299	773
Jardinella accuminata	The tallest spired *Jardinella*	36	46
Jardinella jesswiseae	Hydrobiid snail with an intermediate spire	203	103
Jardinella pallida	A smaller mid-sized hydrobiid	3	13
Gabbia fontana	The only bithyniid snail, with a distinctive white operculum	214	104
Glyptophysa sp.	A distinctive and large planorbid snail	4	2
Gyraulus edgbastonensis	A flat-spired planorbid snail	21	6
Ostracods	Most likely one endemic species (Ponder *et al.* 2010)	17	124
Amphipods	As above	2	39
Shrimp	*Caridina thermophila* is the only known present species	18	1
Fish	*Gambusia holbrooki* is the only fish known from E509	2	0
Insects	Multiple orders (e.g. hemiptera, diptera, coleoptera, odonata)	345	32

twice a day) occurred throughout 2011 and into 2012, to determine the effectiveness of the treatment.

Monitoring of aquatic invertebrates

Monitoring the populations of non-target species (aquatic invertebrates) in areas treated with rotenone was a specific condition placed on the project through the EPBC (1999) referrals process (see Acknowledgements). A method for monitoring aquatic invertebrates was adapted from a previous technique (Munro *et al.* 2009), and a sampling grid was created in Spring E509 (Fig. 2) by marking five longitudinal lines 5 m apart and five transects 3 m apart on each line. Samples were collected at the intersection of each line and transect in November 2010 and February 2011 (before rotenone treatment) and in September 2011 and February 2012 (after rotenone treatment). Each sample comprised a 10-cm sweep of the spring water and substrate, using a small aquarium dip net (10 × 15 cm) at each sample point. Each sample was preserved in a 70% ethanol solution and transported to Brisbane for sorting and identification.

All field samples were sorted and identified using a stereo microscope into 12 categories (Table 1). Categories were selected to minimise the time taken to process multiple samples and prioritise detection of the large number of endemic molluscs present at Edgbaston.

Data analysis – monitoring aquatic invertebrates

Aquatic invertebrate samples from Spring E509 were grouped by line (see Monitoring of aquatic invertebrates above, in Materials and methods), with each line made up of a group of five samples taken at 3-m intervals on each sampling occasion (November 2010, February 2011, September 2011 and February 2012). Assemblage patterns for each sampling occasion were plotted in ordination space by using hybrid non-metric multidimensional scaling (MDS), with the MDS plots generated from Bray–Curtis similarity matrices produced from species presence/absence data. One-way analysis of similarities (ANOSIM) based on the same similarity matrices was used to identify assemblage differences in samples taken on each sampling occasion, and SIMPER was used to describe the main species contributing to the differences between samples taken before and after rotenone treatment. All multivariate analyses were undertaken in the PRIMER version 5 software package (Clarke and Gorley 2001).

Red-finned blue-eye relocation

Genetic studies of the naturally occurring populations of red-finned blue-eye at Edgbaston in 2009 and 2010 indicated that the populations from throughout the complex are genetically mixed, and that relocating groups of fish from the remaining wild populations is unlikely to affect the evolutionary trajectory of the species (Faulks and Kerezsy 2011). Relocation events of red-finned blue-eye occurred in early 2009 and early 2011, in an effort to increase the number of populations. Establishing new populations of red-finned blue-eye in comparatively ‘safe’ springs at Edgbaston remains the only option for increasing the number of populations of the species, apart from captive breeding or *ex situ* relocation (see Discussion). In all relocation events, small groups (20 or fewer) of red-finned blue-eye were sourced from Springs NW30 and NW90n (Fig. 2) and moved to springs where (1) no fish of any species was known to occur, and (2) the chance of the receiving spring being colonised by gambusia was considered small.

In early 2009, populations were relocated to Springs E501, E502, NW72 and E525 (Fig. 2). In early 2011, populations were relocated to E524, E518 and E504 (Fig. 2). Red-finned blue-eye individuals were collected from donor springs by using a small aquarium dip net and kept in aerated water-filled buckets before being released in receiver springs. The time between capture and release was less than 10 min during all relocation operations because the distances between springs at Edgbaston rarely exceed 4 km (Fig. 2).

Following relocation events, the populations of red-finned blue-eye in all donor and receiver springs were monitored

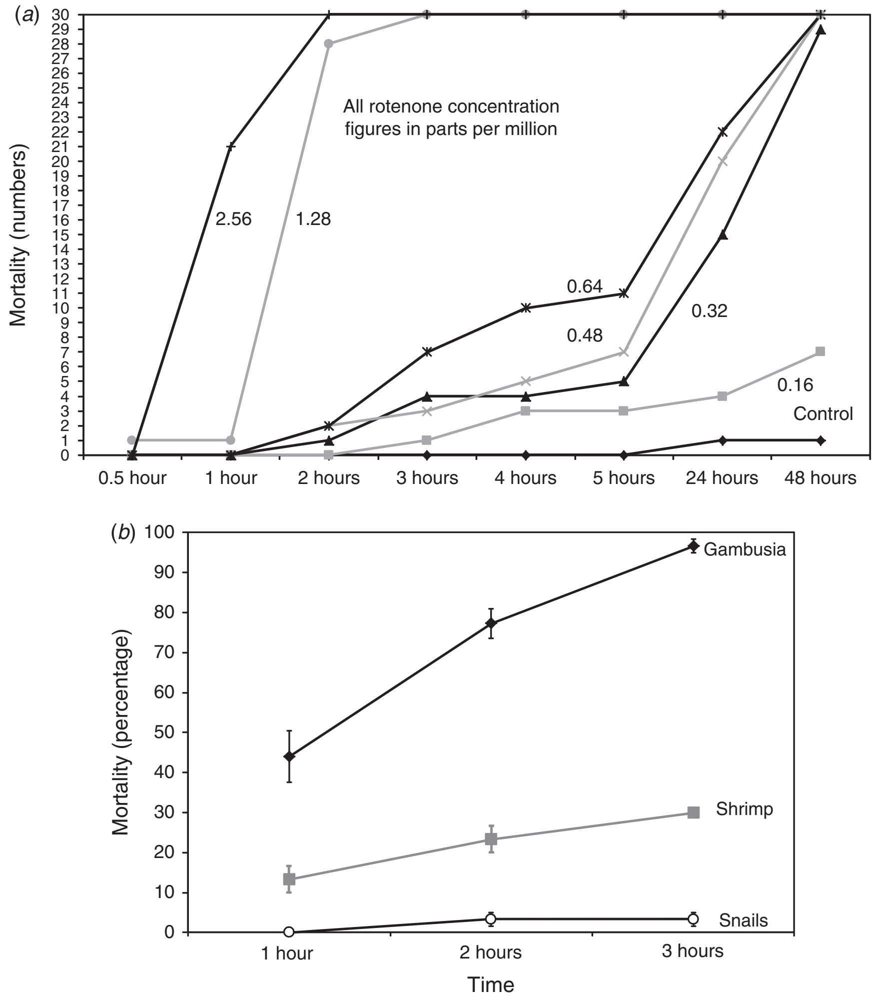

Fig. 4. (*a*) Gambusia mortality for different concentrations of rotenone in a non-replicated experiment conducted in water ≤20°C, and the mortality (mean ± s.e.) for gambusia, shrimp (*Caridina thermophila*) and snails (multiple species) from a tank experiment conducted at Edgbaston by using rotenone at a concentration of 0.32 ppm. (*b*) Water temperature increased from 23.5°C to 25°C from commencement to conclusion of this experiment.

opportunistically whenever the property was visited, by slowly wading through each spring and estimating the number of fish seen. Conventional fish-sampling equipment cannot be deployed in the shallow water of most of the springs at Edgbaston, and visual survey has been demonstrated to be a suitable sampling technique in areas of clear and shallow water, especially if minimising potential harm to endangered species is a priority (Jordan *et al.* 2008).

Results

Gambusia control

Rotenone dosage rate

Rotenone used at all concentrations above 0.16 ppm was effective for killing gambusia in aquaria at water temperatures ≤20°C, and when used in higher concentrations, the time taken to achieve 100% mortality of gambusia was reduced (Fig. 4*a*). Gambusia mortality was 100% within the first 2 h when rotenone was used at 1.28 and 2.56 ppm, and these dosage rates were considered too high for future use at Edgbaston because of the chance of harming non-target organisms. Gambusia mortality was 50% or greater within the first 24 h for dosage rates between 0.32 and 0.64 ppm, and these rates were considered the most appropriate for future gambusia control work because the experiment was undertaken in comparatively cold water (17–20°C) and rotenone has been demonstrated to be more effective as water temperatures rise (Dawson *et al.* 1991; Ling 2003).

The effect of rotenone on aquatic invertebrates and gambusia – tank trials at Edgbaston

Rotenone used at a concentration of 0.32 ppm in water sourced from Spring E509 resulted in >95% mortality of gambusia within 3 h during the in-field tank experiment at Edgbaston when water temperatures ranged between 23.5°C and 25°C (Fig. 4*b*). Mortality of the shrimp, *Caridina thermophila*, was far lower (<30% within 3 h; Fig. 4*b*). There was negligible mortality of all aquatic snail species during the

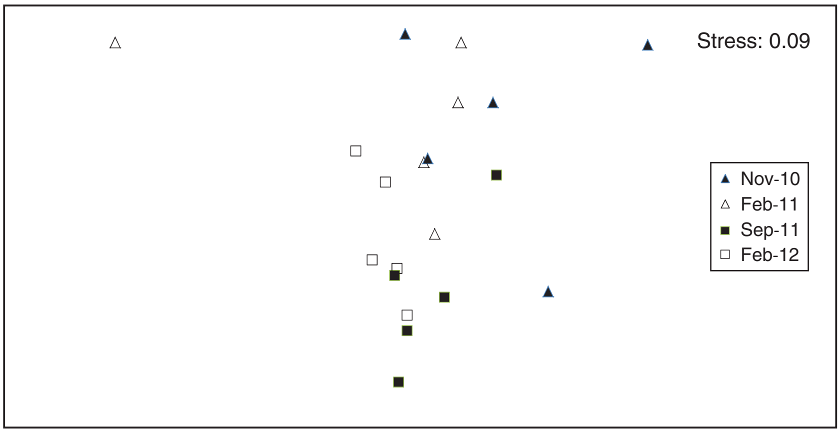

Fig. 5. Two-dimensional non-metric scaling ordination plot of presence/absence data for aquatic invertebrate communities before (triangles) and after (squares) rotenone treatment in Spring E509 at Edgbaston.

Table 2. Paired one-way ANOSIM comparisons of aquatic invertebrate presence/absence from Spring E509 on four sampling occasions and spanning treatment of the spring with rotenone

Pairwise test	Global *R*	*P*
November 2010 (before rotenone) *v.* February 2011 (before rotenone)	0.03	0.294 (n.s.)
November 2010 (before rotenone) *v.* September 2011 (after rotenone)	0.402	0.016
November 2010 (before rotenone) *v.* February 2012 (after rotenone)	0.43	0.008
February 2011 (before rotenone) *v.* September 2011 (after rotenone)	0.276	0.024
February 2011 (before rotenone) *v.* February 2012 (after rotenone)	0.354	0.016
September 2011 (after rotenone) *v.* February 2012 (after rotenone)	0.112	0.183 (n.s.)

experiment (Fig. 4*b*). A rotenone dosage rate of 0.32 ppm was chosen for subsequent rotenone trials in springs because it was effective for killing the majority of gambusia within 3 h in water temperatures similar to those in other springs at Edgbaston. Higher dosage rates were not considered because the tank trial indicated that rotenone at 0.32 ppm may have an adverse impact on shrimp.

Results from rotenone application in a spring, February to September 2011

An estimated 70% mortality of gambusia occurred during the initial rotenone treatment at Spring E509 (two treatments at 0.32 ppm over consecutive days) in February 2011; however, difficulties in achieving full coverage of wet areas were encountered because of the amount of spring vegetation (see Rotenone application in a spring above, in Materials and Methods).

The repeated treatment in May 2011 (after reducing the spring vegetation) was more effective, with no gambusia observed during subsequent monitoring in either June or July. In August 2011, after three visual monitoring surveys, five gambusia individuals were observed in Spring E509 and rotenone was again applied on consecutive mornings at 0.32 ppm. In September 2011, the spring was inspected for 30 min on three separate monitoring occasions. No gambusia were observed on either monitoring occasion, and the spring was treated once more with rotenone as a precautionary measure. In November 2011, Spring E509 was inspected for 30 min on three separate monitoring occasions and no gambusia were detected.

Despite heavy rain during the summer period 2011–2012, which was expected to facilitate gambusia colonisation of Spring E509, no gambusia were detected in February 2012 after 60 min of visual survey.

Populations of non-target species before and after rotenone treatment in Spring E509

All invertebrate species and taxa sampled in Spring E509 before rotenone application were also present after rotenone application (Table 1). However, analysis of invertebrate presence/absence revealed a separation of communities before and after rotenone application (Fig. 5). There was a significant difference in the presence/absence of invertebrate species in samples taken from November 2010 to February 2012 (Global R: 0.248, $P = 0.002$); however, this was due to differences between all before- and after-rotenone samples rather than by differences within the before and after groups (Table 2). The results indicate that the composition of aquatic invertebrate communities in springs such as E509 is likely to vary through time and that determining the precise effect of rotenone application may be difficult.

Common invertebrate species with a widespread distribution within Spring E509 (such as the hydrobiid snails *Jardinella edgbastonensis*/*corrugata*, *Jardinella jesswiseae* and *Jardinella*

Table 3. SIMPER results for species contributing >5% to similarity within and/or difference between presence/absence of aquatic invertebrates from samples taken before and after rotenone treatment at Spring E509
AS = average similarity, AD = average dissimilarity

Species	Before rotenone (AS 71.4%)	After rotenone (AS 85.68%)	Before *v.* after (AD 27.41%)
J. edgbastonensis/corrugata	20.3	16.01	–
Insect	16.25	16.01	–
Jardinella jesswiseae	14.52	16.01	–
Gabbia fontana	14.52	16.01	–
Ostracod	11.15	16.01	6.52
Jardinella accuminata	6	9.47	11.96
Gyraulus edgbastonensis	11.34	–	17.14
Amphipods	–	6.86	16.43
Jardinella pallida	–	–	12.61
Shrimp	–	–	12.23
Glyptophysa sp.	–	–	7.99

accuminata, the bithyniid snail *Gabbia fontana*, ostracods and insects) made similar proportional contributions to both before-rotenone and after-rotenone samples (Table 3), whereas dissimilarity was largely driven by species sampled in smaller numbers that were concentrated in either the before-rotenone samples (such as the planorbid snail, *Gyraulus edgbastonensis*) or the after-rotenone samples (such as amphipods; Tables 1, 3). Total numbers of common species (notably the *Jardinella edgbastonensis/corrugata* snail group) increased in post-rotenone samples, whereas this result was reversed for insects (Table 1). There was an expected bias in the sampling method towards benthic invertebrates (such as snails, ostracods and amphipods) and more mobile animals such as shrimp (and gambusia) were rarely collected, despite being observed during visual survey (Table 1).

Red-finned blue-eye relocation 2009–2012

A comparatively small number of individual red-finned blue-eyes (maximum 20) was relocated to each of seven receiver springs in trials between 2009 and 2012, to assess survival in new habitat, while minimising potential harm to donor populations. Of the seven trials, three have been successful (fish are currently present), three have failed (fish are absent and presumed extirpated) and in one case (Spring E504), the relocated individuals were later removed as a precaution when gambusia were detected in an adjacent ephemeral creek (see Figs 2, 3).

The red-finned blue-eye population that was relocated to Spring NW72 in April 2009 had demonstrated recruitment in the new habitat by October 2009. This population has continued to recruit in the intervening period (2009–2012) and is currently considered a self-sustaining relocated population as a result. The relocated population in Spring E524 also bred by February 2012, whereas the population in Spring E502 has remained self-sustaining (although small in number) since relocation in early 2009.

The population relocated to Spring E501 also appeared to breed successfully in late 2009, but later became extirpated during the 2009–2010 summer. The population in E525 persisted for approximately two years following relocation but became extirpated in early 2011.

The population that was relocated to Spring E518 in early 2011 appears to have failed as no fish have been observed since August 2011. The fish relocated to Spring E504 were later re-captured and moved back to NW30 in September 2011, following detection of gambusia in an adjacent creek (to avoid risking the loss of the relocated fish through gambusia interaction).

Discussion

Gambusia control

Removing gambusia from springs at Edgbaston is necessary to create pest-free habitat for the re-establishment of red-finned blue-eye populations. By doing so, the risk of species extirpation can be reduced by ‘spreading the risk’ among multiple spring locations. Unlike many connected riverine systems, the isolated nature of the waterbodies at Edgbaston provides opportunities for effecting such localised pest eradication.

Despite the mixed success of rotenone as a method for eradication of pest fish such as gambusia (Pyke 2008), the results from this work at Edgbaston indicate that in small, shallow isolated areas where effective treatment is feasible, piscicides are a viable method for control (Lintermans and Rutzou 1990; Rayner and Creese 2006). Similar work in England removing topmouth gudgeon, *Pseudorasbora parva* (Allen *et al.* 2006), and in the United States removing northern snakehead, *Channa argus* (Lazur *et al.* 2006), also demonstrated the effectiveness of rotenone as an effective means of controlling pest fish species.

At Edgbaston, rotenone can be applied by one operator using either a vehicular or backpack spray unit. Preparation of the proposed treatment area (such as reduction of vegetation to minimise areas where the chemical cannot disperse) will increase the effectiveness of the treatment, and the use of a marker dye is essential to show where the spray has been applied. Repeated treatments over several months are likely to be necessary to eradicate gambusia from such areas, and repeated and vigilant monitoring is crucial to identify re-colonisation after treatment and assess suitability for re-introduction of red-finned blue-eye.

Constantly discharging springs, such as those at Edgbaston, present a challenge with regard to calculating dosage rates, because in such a dynamic environment, dilution of the rotenone product occurs quickly in or around spring vents. Although the dosage rate used in the trial Spring E509 (0.32 ppm) was adequate for killing gambusia within 1–2 h of application in areas of open water, extra chemical (such as the last 10 L of each spray tank) had to be applied to the spring vents to ameliorate this dilution effect. Future eradication attempts could trial higher dosage rates to increase the effectiveness of rotenone over a shorter time period, and thus reduce the number of times target springs need to be treated. It is anticipated that eradicating gambusia will be substantially more difficult in larger springs where dilution effects are likely to be greatest (such as those in the north-eastern section of the complex that were colonised by gambusia before 2008).

Liquid rotenone may be an alternative to powdered chemical that would not clog delivery equipment; however, testing the effects of emulsifiers on non-target species would need to be undertaken before any spring trials. Other chemicals and anaesthetics such as tricaine methanosulfate, quinaldine, benzocaine, metomidate hydrochloride, clove oil and Aqui-S may also be suitable for gambusia-removal activities at Edgbaston into the future (see Munday and Wilson 1997; Griffiths 2000; Small 2003), although none is currently registered for pest fish control in Australia.

Work in England on removal of the topmouth gudgeon (Britton and Brazier 2006), and in Australia on the removal of rainbow trout (Lintermans 2000; Lintermans and Raadik 2001), has demonstrated that application of a chemical such as rotenone must be accompanied by strategies to prevent re-colonisation. Methods of preventing re-colonisation of springs at Edgbaston, using techniques such as barriers made from garden edging material, silt fencing and excavated earth, are currently under investigation.

Rotenone and non-target organisms

Determining the effect of rotenone treatment on non-target organisms has been a primary goal of the gambusia control program at Edgbaston to-date. Studies demonstrate that the chemical can be lethal to non-target organisms such as invertebrates and amphibians (Anderson 1970; Chandler and Marking 1982; Vehovszky *et al.* 2007).

The aquatic invertebrate monitoring undertaken at Edgbaston before and after rotenone application in Spring E509 suggested that in wetlands of this type (sustained by the Great Artesian Basin), most resident aquatic invertebrates are either unaffected by, or recover comparatively quickly from, sporadic rotenone treatment to control gambusia. A possible exception is the small shrimp, *Caridina thermophila*, and more robust experimental studies on the toxicity of rotenone to this species are warranted (especially if rotenone concentrations are increased in future management efforts). In general, studies relating to rotenone toxicity on crustaceans are uncertain; marine shrimps appear susceptible (Bussing 1972; Lockett 1998); however, Ogunsanya *et al.* (2011) concluded that the freshwater prawn, *Macrobrachium rosenbergii*, should be able to tolerate rotenone concentrations required to kill pest fish in aquaculture, and Gehrke (2001) demonstrated that three species of decapod crustaceans are unlikely to be affected by pelleted rotenone designed to control large-bodied invasive fish such as carp.

The results of the before-and-after rotenone sampling also demonstrated that populations of aquatic invertebrates may fluctuate widely, and that some species (such as the snails *Jardinella pallida* and *Glyptophysa* sp.) may be uncommon for extended periods (or within a particular spring). Sampling along a longer temporal scale may be necessary to more accurately assess the distribution and resilience of resident biota. As an example, the populations of most snail species, amphipods and ostracods all increased in the samples following rotenone treatment, but whether this is due to gambusia removal or other factors is undetermined. The targeted application of rotenone to specific areas within Spring E509 may have contributed to this positive result for invertebrates, because the chemical was applied only to habitat within 6 m of the spring vent (the area where open water was present). It is possible that fauna susceptible to rotenone suffered local extirpation in these areas but that populations re-colonised from surrounding areas of the spring shortly afterwards. It is also possible that rotenone became non-harmful within a few hours of application as a result of constant dilution from the spring vents, and/or that it degraded quickly (as water temperatures at Edgbaston rarely fall below 24°C close to the spring vents).

Evidence gathered from the present study indicates that selective rotenone application in Australian springs should be further pursued as a method of removing pest fish that does not cause long-term harm to resident non-target organisms if tested and applied carefully. During future rotenone work at Edgbaston before and after monitoring of aquatic invertebrates is recommended (as is the selective approach to spray application outlined above). Monitoring non-target species in non-treatment springs would also be beneficial because it would facilitate temporal comparison of natural *versus* rotenone-affected variation of these populations.

Red-finned blue-eye – population recovery options

The declining populations of red-finned blue-eye at Edgbaston (Fig. 3) present an urgent ecological problem to managers, researchers and government agencies charged with the care of Australia's endangered species. The progressive colonisation of springs by gambusia, and the localised extirpation of red-finned blue-eye that inevitably follows, suggest that the last populations of the endangered species will be lost without intervention. This could represent the first documented extinction of a freshwater fish in Australia in modern times. The colonisation of one of the last remaining springs containing a naturally occurring population of red-finned blue-eye by gambusia (Spring NW90n during summer 2010–2011) is a poignant example of the urgency that surrounds the conservation efforts at Edgbaston.

Recovering red-finned blue-eye populations is contingent on preserving all remaining extant populations and establishing new populations in additional 'safe' springs where gambusia is absent. However, and as demonstrated above, the success of such management interventions is likely to be highly variable. As an added complication, there are fewer than 10 springs within the Edgbaston complex that could currently be considered 'safe' from possible gambusia colonisation, and all of these have already been used in relocation trials. There are currently no more receiver springs within the complex that could be considered 'safe' for red-finned blue-eye relocation.

Despite the scarcity of currently suitable sites, relocation of red-finned blue-eye within the spring complex at Edgbaston remains the most sensible solution for *in situ* species recovery. This will be contingent on successful eradication of gambusia from springs, with suitable habitat and subsequent 'quarantine' of these springs to prevent further colonisation. Results from red-finned blue-eye relocation effort since the 1990s indicate that this method is appropriate, although success has been variable (Fig. 3). Furthermore, it is salient to note that establishment of self-sustaining populations does not equate to long-term persistence (Seddon 1999), and that earlier relocations in the south-western spring group at Edgbaston (SW50, SW60 and SW70) almost certainly became locally extirpated because of invasion by gambusia.

Given that rotenone has been demonstrated to be a successful method of eradicating gambusia, a long-term (10-year) program has been proposed (Kerezsy 2012*b*) that aims to remove this species from potential springs that may be suitable as red-finned blue-eye 'sanctuaries' into the future. A quarantine option currently being trialled at Edgbaston involves the installation of an earthen bund around springs that contain two of the last naturally occurring populations of red-finned blue-eye. Although this compromises the ability of spring fauna to migrate between springs during overland flow episodes, it may result in comparatively 'safe' populations of red-finned blue-eye that are protected from gambusia invasion in the future. Precedents exist for such an approach in wildlife recovery, and examples include predator-proof fences to protect populations of Hamilton's frog, *Leiopelma hamiltoni*, in New Zealand (Brown 1994) and marsupials such as the greater bilby, *Macrotis lagotis*, and burrowing bettong, *Bettongia lesueur*, in South Australia (James and Eldridge 2007).

The more recent relocations of red-finned blue-eye (2009 and 2011) have contributed some useful insights into the success of this technique. The most successful relocation (at Spring NW72) resulted from a donor population of 20 in April 2009 and has consistently numbered many hundreds in the ensuing years. A similarly positive result is evident in Spring E524 from April 2011, where the small donor population (20) began breeding shortly after relocation. This indicates that suitable habitat may be the most important factor influencing the persistence of relocated populations, but also raises concerns regarding the genetic adequacy of such a small founder population.

In contrast, the failure of relocated populations at both Spring E501 in 2009 and Spring E518 in 2011 may be because these relocations occurred in springs where there was an abundance of water but the spring habitat (such as an absence of shallow areas and emergent plants) was very different. Sheller *et al.* (2006) analysed 148 translocations of the endangered Gila topminnow, *Poeciliopsis occidentalis*, and found that season, habitat and genetic origin all affected the persistence time of stocked populations, so it is equally likely that relocations of red-finned blue-eye made at a different or repeated time may produce a more successful outcome or that donor populations of 20 may be too small on some occasions. Given that relocation has been demonstrated to be partially successful, it would be prudent to experiment with larger donor-population sizes and monitor results. However, removal of larger numbers of red-finned blue-eye from naturally occurring populations at Edgbaston is problematic because these populations continue to decline.

Maintaining populations of endangered species in similar but geographically separated environments is a frequently employed recovery technique, and this has certainly been demonstrated as a successful method in the case of the Australian freshwater fish Pedder galaxias, *Galaxias pedderensis*, in Tasmania (Allen *et al.* 2002; Chilcott *et al.* 2013). The degree to which such an approach could be successful for red-finned blue-eye has not yet been investigated *ex situ*. However, given the constant threat of gambusia colonisation at Edgbaston, the establishment of such populations in other areas should be considered. In the Queensland Lake Eyre Basin, Great Artesian Basin spring complexes with similar water quality but (as yet) no gambusia occur at Elizabeth Springs in the Diamantina catchment and in the eastern Simpson Desert in the Mulligan catchment (A. Kerezsy, pers. obs.).

Captive breeding of endangered fish species is a commonly employed method for species preservation (Philippart 1995), with the caveat that it should be used as a last resort and only when other avenues for extinction amelioration have been exhausted (Ellis *et al.* 2011). Captive breeding has certainly been undertaken for many endangered fish species from arid areas in the south-western United States (Johnson and Jensen 1991) and, in Australia, examples of captive breeding of endangered animals for re-introduction to the wild include fish such as the Murray hardyhead, *Craterocephalus fluviatilis* (Ellis *et al.* 2013), purple-spotted gudgeon, *Mogurnda adspera*, and Yarra pygmy perch, *Nannoperca obscura* (Hammer *et al.* 2013; Saddlier *et al.* 2013). Captive breeding of red-finned blue-eye has not been undertaken in the time since the fish was listed as *Endangered*, and no captive populations have endured from the time when hobbyists kept the species (Fairfax *et al.* 2007). It is possible that failure to replicate the unusual environmental conditions that exist at Edgbaston has contributed to the failure of captive populations. Nevertheless, the declining populations of red-finned blue-eye in the wild and the fact that these populations are at risk of colonisation by gambusia combine to suggest that establishment of a captive population or populations is now a priority, and experienced aquarists, aquaculturists and fisheries biologists are willing to assist such a venture (Peter Unmack, Brendan Ebner, Dave Wilson, Michael Hammer, Steve Brooks, Leo O'Reilly, Iain Ellis, pers. comm.).

Legislative considerations

Like Canada and the United States, Australia has a federal system of government that oversees individual state and territory jurisdictions, and different endangered species legislation is enacted and enforced at both levels (Goble *et al.* 1999). When a species (such as the red-finned blue-eye) is listed as endangered under both federal and state legislation, statutory approval of projects such as those discussed above must be gained within the different jurisdictions, and this can create a considerable time lag between the initiation of a project and its execution. As an example, permission to undertake the rotenone trials on gambusia and red-finned blue-eye relocation at Edgbaston required both a referral under the federal *EPBC Act* (EPBC 1999) and the granting of a Scientific Purposes Permit under the relevant Queensland endangered species legislation (NCA 1992), a process that took over 12 months. It is likely that approval to conduct future work, such as more extensive rotenone treatment or a captive-breeding program, may take a similar time. These delays may be significant with regard to the urgency of red-finned blue-eye decline, because it is conceivable that populations of red-finned blue-eye may be extirpated by gambusia colonisation within the timeframe of the legislative approvals process.

The plight of the red-finned blue-eye provides an opportunity for state and federal government agencies to work co-operatively with a conservation organisation to achieve a common desired outcome. Initiatives such as the development of a captive-breeding agreement with the Queensland Threatened Species Committee and notification of staff within the federal agency have commenced, and it is hoped that this co-operative approach may deliver benefits in streamlining some of the legislative requirements associated with the conservation of red-finned blue-eye.

Conclusion

The gambusia control and red-finned blue-eye recovery project at Edgbaston has demonstrated some habitat-specific techniques that may be useful in preventing extirpation of an endangered species, but a longer-term project is now required to create gambusia-free springs to serve as 'safe' red-finned blue-eye habitat in the future, as well as pursuing other strategies such as captive breeding.

The results demonstrate that not all recovery efforts for the red-finned blue-eye will be successful, and conservation management should acknowledge that some activities will fail. Most importantly, however, the trials since 2009 have demonstrated that careful application of rotenone can be a successful method for eradicating gambusia from certain springs, and that targeted relocation of red-finned blue-eye can be a successful method for establishing additional populations of an endangered species that is close to extinction.

The intent of Bush Heritage Australia is to conserve not only the red-finned blue-eye but the entire suite of biota that is present at, and mostly endemic to, the unique springs at Edgbaston. We encourage state and federal natural-resource agencies to work cooperatively with the company to achieve this goal. Given the geographic isolation of Edgbaston and the physical separation of the springs, the opportunities for meaningful conservation at the site are promising. The fact that the property is privately owned and managed by a conservation company, and the strong body of research and on-ground actions that have been conducted (and outlined in the present paper) combine to indicate that further investment towards continuing this conservation program seems prudent.

The most compelling argument for attempting to save the red-finned blue-eye is the certainty that failure to try will inevitably result in the extinction of another Australian vertebrate, and the first known extinction of an Australian freshwater fish.

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