









RESEARCH ARTICLE

Alternative conservation outcomes from aquatic fauna translocations: Losing and saving the Running River rainbowfish

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Abstract

1. The translocation of species outside their natural range is a threat to aquatic biodiversity globally, especially freshwater fishes, as most are not only susceptible to predation and competition but readily hybridize with congeners.
2. Running River rainbowfish (RRR, *Melanotaenia* sp.) is a narrow-ranged, small-bodied freshwater fish that recently became threatened and was subsequently listed as Critically Endangered, owing to introgressive hybridization and competition following the translocation of a congeneric species, the eastern rainbowfish (*Melanotaenia splendida*).
3. To conserve RRR, wild fish were taken into captivity, genetically confirmed as pure representatives, and successfully bred. As the threat of introgression with translocated eastern rainbowfish could not be mitigated, a plan was devised to translocate captive raised RRR into unoccupied habitats within their native catchment, upstream of natural barriers. The translocation plan involved careful site selection and habitat assessment, predator training (exposure to predators prior to release), soft release (with a gradual transition from captivity to nature), and post-release monitoring, and this approach was ultimately successful.
4. Two populations of RRR were established in two previously unoccupied streams above waterfalls with a combined stream length of 18 km. Post-release monitoring was affected by floods and low sample sizes, but suggested that predation and time of release are important factors to consider in similar conservation recovery programmes for small-bodied, short-lived fishes.

KEYWORDS

Australia, Burdekin, captive breeding, conservation, freshwater, hybridization, *Melanotaeniidae*, threatened species

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1 | INTRODUCTION

The translocation of alien species is a major threat to many ecosystems worldwide (Vitousek et al., 1997; Clavero & García-Berthou, 2005; Gallardo et al., 2016). Globally, the rate of translocations has been increasing (Seebens et al., 2017), with alien species currently present on every continent (Prins & Gordon, 2014). Although there has been considerable research examining the adverse effects of alien species (McNeely, 2001; Prins & Gordon, 2014), translocations can also be an effective tool for conservation and management (Minckley, 1995; Tuberville et al., 2005; IUCN/SSC, 2013). Translocation has become a key tool for conserving freshwater fishes, using both wild and captive-bred fishes (Minckley, 1995; Lintermans, 2013a; Lintermans et al., 2015). When referring to different types of conservation translocations, this article follows the definitions provided by the International Union for Conservation of Nature Species Survival Commission (IUCN/SSC, 2013).

Most early conservation translocations of fish have involved large-bodied threatened species that were often potential angling targets (Minckley & Deacon, 1991; Lintermans et al., 2015). However, the practice has also been applied to smaller threatened fishes (Minckley & Deacon, 1991; Hammer et al., 2013; Lintermans et al., 2015; Tatár et al., 2016). The continued existence of certain species, such as the Pedder galaxias (*Galaxias pedderensis*) is solely the result of conservation translocations (Chilcott et al., 2013), whereas the conservation status of several Critically Endangered species, such as the red-finned blue-eye (*Scaturiginichthys vermeilipinnis*; Kerezszy & Fensham, 2013) and several other galaxiid species (Koster, 2003; Hardie, Barmuta & White, 2006; Ayres, Nicol & Raadik, 2012) have benefited substantially from translocations.

A review of factors influencing the success of freshwater fish reintroductions reported that second to addressing the cause of initial decline, habitat-related factors were the greatest predictors of reintroduction success (Cochran-Biederman et al., 2015). The importance of suitable habitat in determining the success or failure of conservation introductions is echoed by studies of invasive fish species, which have found that if the habitat characteristics of the receiving environment are suitable then an invasion is likely to succeed, regardless of other factors (Moyle & Light, 1996a; Moyle & Light, 1996b; Harris, 2013). That an introduction is likely to fail in the absence of suitable habitat seems straightforward; however, some reintroductions may fail even in the presence of adequate habitat (Barlow, Hogan & Rodger, 1987; Leggett & Merrick, 1997).

Out of all failed conservation translocations of fish, 71% used captive-reared fish (Cochran-Biederman et al., 2015). Captive-reared fish are often raised under conditions that are vastly different from the environment into which they are released (Brown, Davidson & Laland, 2003). Consequently, captive-reared fish often exhibit behaviours that are detrimental to their survival in the wild, and as a result often suffer from high mortality rates once released (Brown & Day, 2002; Ebner, Thiem & Lintermans, 2007; Sparrevohn & Støttrup, 2007), which is a prevalent problem across fauna groups (Berger-Tal, Blumstein & Swaisgood, 2020). The behavioural impacts

of captive rearing have been known for some time (Brown & Day, 2002), with captive-reared fish showing deficiencies in key behaviours such as predator recognition and avoidance (Alvarez & Nicieza, 2003; Ebner, Thiem & Lintermans, 2007) and foraging skills (Brown & Laland, 2002; Brown, Davidson & Laland, 2003). Studies on the success of conservation introductions of freshwater fishes within Australia (Ebner, Thiem & Lintermans, 2007; Ebner, Johnston & Lintermans, 2009; Brown et al., 2012) and abroad (Alvarez & Nicieza, 2003) suggest that predation and competition are likely to play a major role in translocation success. Brown, Davidson & Laland (2003) showed that environmental enrichment and exposure to live foods resulted in fish being better able to handle novel prey items. Meanwhile, several studies have shown that repeated exposure to predators, or their stimulus (e.g. scent or pictures), will improve the predator avoidance behaviours of captive-bred fish (Brown, 2003a; Vilhunen, 2006; Hutchison et al., 2012; Abudayah & Mathis, 2016). As a result, research and implementation of environmental enrichment and predator training of captive-reared fish is becoming more commonplace (Vilhunen, 2006; Hammer et al., 2012; Roberts et al., 2014; Lintermans et al., 2015).

Most research investigating methods to improve the survival of captive-reared fishes has taken place overseas, although some recent research has been conducted in Australia (Hutchison et al., 2012). In both cases, the research investigating introduction success has focused almost entirely on large-bodied, predatory, recreationally important species, such as brown trout (*Salmo trutta*) and rainbow trout (*Oncorhynchus mykiss*) (Brown & Smith, 1998; Alvarez & Nicieza, 2003; Brockmark, Adriaenssens & Johnsson, 2010), or percichthyids (Ebner, Thiem & Lintermans, 2007; Ebner & Thiem, 2009; Hutchison et al., 2012). However, of the 17 Australian species used in conservation introductions documented by Lintermans et al. (2015), 10 were small-bodied species. Small-bodied species usually have vastly different requirements compared with large-bodied species, and a conservation measure that works well for large species may not be as effective for smaller species (e.g. growing them to a large size to prevent predation).

1.1 | Study organism background

The extinctions and declines of native fishes resulting from hybridization with alien species have been well documented throughout Europe and North America (Hitt et al., 2003; Rosenfield & Kodric-Brown, 2003; Meldgaard et al., 2007; Ludwig et al., 2009). Compared with other countries, introgressive hybridization with alien species has not typically been considered a threat to Australia's native biodiversity (Hitt et al., 2003; Meldgaard et al., 2007; Ludwig et al., 2009) because most alien species have originated from other continents with biota that are taxonomically distant (Lintermans, 2013a). However, high levels of genetic structuring between populations as well as many new cryptic species were identified by recent broadscale genetic studies of Australian freshwater fishes (Hammer et al., 2007; Raadik, 2014; Shelley

et al., 2018). Accordingly, introgressive hybridization caused by translocations of 'native' species outside their natural range, or from one part of a species range to another, has more recently been recognized as a threat to conservation for Australian freshwater fishes (Lintermans et al., 2005; Harris, 2013; Couch et al., 2016).

Endemic to Australia and New Guinea, the family Melanotaeniidae, or rainbowfishes, contains more than 110 species with multiple undescribed taxa (Unmack, Allen & Johnson, 2013). The genus *Melanotaenia* is by far the most numerous and widespread in Australia, occurring throughout the northern half of the continent and into south-eastern regions (Unmack, Allen & Johnson, 2013). There are several 'lineages' within the genus, and species within the same lineage rarely co-occur (Unmack, Allen & Johnson, 2013). In 2016, the Australian Society for Fish Biology (ASFB) listed four *Melanotaenia* species as Vulnerable, Endangered, or Critically Endangered, owing to introgressive hybridization with a widespread member of the genus (Lintermans, 2016), with a subsequent International Union for Conservation of Nature (IUCN) assessment confirming their threatened status (Hammer, Unmack & Brown, 2019b).

One of the species listed by the ASFB and the IUCN was the Running River rainbowfish (RRR *Melanotaenia* sp.). This species was first recorded in 1981 as a phenotypically unique population of rainbowfish from the usual native eastern rainbowfish (*Melanotaenia splendida splendida*) found in most rivers in the region (Martin & Barclay, 2016). Further collections across the region suggested that there was a complex of different rainbowfish populations, the

taxonomy of which was unclear (Martin & Barclay, 2016). As part of a broader rainbowfish research project, fieldwork was conducted across the Burdekin River basin in August 2015 to try to resolve the taxonomic status of the various rainbowfish populations native to the region. During this fieldwork it was discovered that eastern rainbowfish had colonized the reach of Running River containing RRR, as well as being established in large numbers further upstream at Hidden Valley (Unmack & Hammer, 2015), an area previously lacking any rainbowfish (Martin & Barclay, 2016). It is unclear whether this represents a new translocation, or whether it represents downstream dispersal from earlier recorded translocated populations above Paluma Dam (although recent searches above Paluma Dam have failed to find any rainbowfish) (Martin & Barclay, 2016). Subsequent genetic and morphological examination supports the recognition of RRR as a separate species (P. Unmack, M. Hammer, G. Allen, unpublished data). As currently recognized, RRR is restricted to 13 km of Running River between two gorges (Figure 1). Running River is a major tributary to the Burdekin River, one of Australia's larger river basins, situated on the north-eastern coast of Queensland (Pusey, Arthington & Read, 1998). The lower gorge prevents the upstream movement of eastern rainbowfish, whereas the upper gorge prevents the movement of RRR further upstream.

Once eastern rainbowfish had been detected in Running River above the upper gorge in 2015 it was realized that RRR was at risk of extinction via hybridization, as no members of the Australis lineage (Unmack, Allen & Johnson, 2013) of rainbowfishes are ever found in

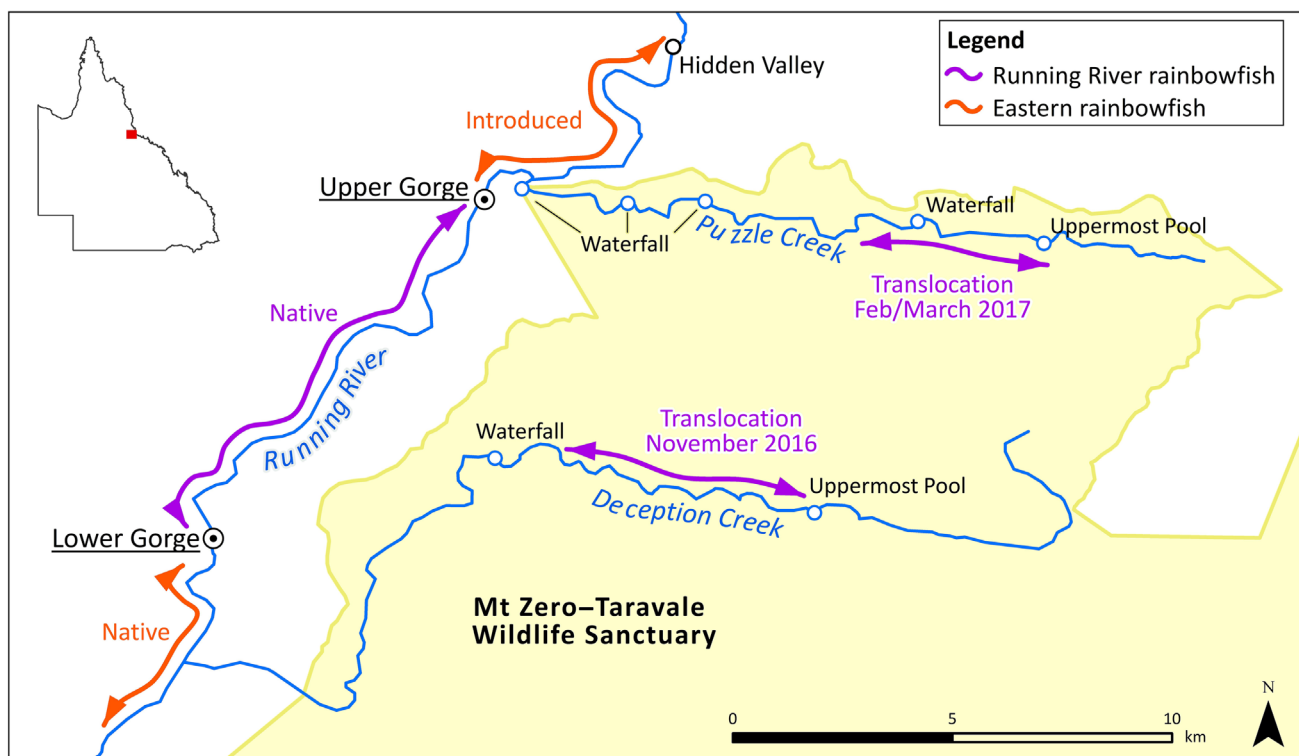


FIGURE 1 Map of the study area showing the location of Puzzle and Deception creeks and their positions relative to Running River and its gorges. Purple arrows indicate the range of Running River rainbowfish, whereas orange arrows indicate the range of the eastern rainbowfish (*Melanotaenia splendida*). Created by AWC Spatial Officer Tani Cooper, and used with permission from the Australian Wildlife Conservancy.

sympatry. At this point it was apparent that this population was distinct and worth conserving, but its taxonomic status would not be clear until genetic work had been conducted. Initially, 52 live wild fish were collected and then brought back to the University of Canberra as an insurance population. As this research lacked any formal funding, crowdfunding was initiated via the University of Canberra Foundation to cover the costs of genotyping potential broodstock, and keeping, breeding, and shipping the fish, and used internal University of Canberra funding to fund a postgraduate research project. Funds were sought by directly contacting various aquarium societies, primarily from North America, Australia, and Europe, as well as being solicited from members of the Australia New Guinea Fishes Association during presentations and in their journal *Fishes of Sahul*. In addition, we put out calls for donations via social media in various Australian native fish-related Facebook groups and in the aquarium magazine *Amazonas*. There is tremendous worldwide interest in rainbowfishes from aquarium hobbyists, as they are brightly coloured and easy to keep and breed. Many aquarium hobbyists, clubs, and businesses have a strong conservation ethos and are enthusiastic about supporting projects like ours by donating money. Once preliminary data on the taxonomy of rainbowfishes in the Burdekin River basin had been collected it became clear that RRR was a unique taxon from the Australis lineage and action was needed to save it.

The only conservation options available for RRR were either to hold the fish in captivity for the long term or to find locations where they could be translocated to, as it would take a massive effort to remove the eastern rainbowfish from upper Running River and then restore RRR in their native range. Maintaining the species in wild habitats was the most feasible option, thus the next challenge was to determine whether any suitable sites for translocation might exist.

The eastern rainbowfish is a capable disperser, occupying most habitats throughout its range, unless there are significant barriers to prevent movement, and thus finding habitats where it is absent is unusual. The region around Running River is seasonally arid and most small creeks in the region do not hold water permanently. The middle to lower section of Running River has two larger tributaries, Deception Creek and Puzzle Creek, which are located on Mount Zero–Taravale (Figure 1), covered by two pastoral leases owned and managed by the Australian Wildlife Conservancy (AWC, a not-for-profit conservation organization). Both creeks have sections that flow through gorges or rocky reaches that hold permanent water, and both were reported to have fishes of unknown species present (T. White, manager of the AWC Mount Zero–Taravale Sanctuary). Both creeks were sampled in February 2016, with Deception Creek having a large population of spangled perch (*Leipotherapon unicolor*), as well as a few purple spotted gudgeon (*Mogurnda* sp.), whereas Puzzle Creek had the same species, but the uppermost section above a waterfall only contained an abundant population of purple spotted gudgeon. Deception Creek flows into Running River below the lower gorge, with eastern rainbowfish native to its lower reaches. One medium-sized waterfall was located on Deception Creek approximately 12 km upstream from the confluence with Running

River. Puzzle Creek flows into Running River in the middle of the upper gorge, which historically probably lacked rainbowfish; in addition, it has several major waterfalls of 10–20 m in height present along its course. As both creeks lacked rainbowfish they were considered suitable long-term translocation sites. This was an extraordinarily fortuitous situation given the lack of permanent streams in the area and the lack of eastern rainbowfish in both these streams. Any translocations into other rivers would have had impacts on native rainbowfish populations located in downstream reaches, whereas the eastern rainbowfish in lower Running River already had a potential influx of RRR from upstream.

As small-bodied freshwater fishes commonly have a high risk of extinction (Reynolds, Webb & Hawkins, 2005; Olden, Hogan & Zanden, 2007; Kopf, Shaw & Humphries, 2017; Lintermans et al., 2020), there is a need for a better understanding of the factors influencing, and methods for improving, the survival of captive-bred small-bodied freshwater fishes once released, to reduce the chance of failure. One example of this type of failure is the previous attempts to return *Melanotaenia eachamensis* (Lake Eacham rainbowfish) to Lake Eacham after they were extirpated owing to the introduction of other fishes (Barlow, Hogan & Rodger, 1987). A captive breeding programme was established (Barlow, Hogan & Rodger, 1987) that produced 3,000 fish, which were then released into the lake; however, subsequent surveys failed to detect any survivors (Brown et al., 2012). Subsequent research showed that captive rainbowfish can behave very differently from wild fish (Brown & Warburton, 1997; Brown & Warburton, 1999a; Kydd & Brown, 2009). This highlights the complexity that can be involved in obtaining successful reintroduction outcomes.

The main goals of the present study were to initiate a conservation programme for a recently recognized, undescribed, small-bodied rainbowfish, the Running River rainbowfish (RRR, *Melanotaenia* sp.). This was achieved through the design and implementation of a conservation strategy that used captive breeding and translocations to conserve the species and to evaluate the success of the strategy to inform future efforts. The study also documented the history of the species, the discovery of the translocation of eastern rainbowfish, and how crowdfunding was used to support the project. This article reports on the results of experiments conducted to examine the role of predator training on translocation success. However, these can be difficult to assess because of the limited replication, small sample sizes, and perturbations caused by weather events.

2 | METHODS

2.1 | Captive breeding

In 2015, 52 RRR were collected from Running River and transported to the University of Canberra. Broodstock were genotyped using single nucleotide polymorphisms (SNPs) based on DNA from fin clips and compared with wild fish that had been collected and preserved in

liquid nitrogen in 1997 (18 years earlier), to ensure genetic purity. These fish were set up as 26 breeding pairs and used as broodstock for Deception Creek releases. In February 2016 additional wild fish were collected, with 32 fish genotyped and added as broodstock for Puzzle Creek releases. Fish were spawned in 17 groups of two males and two females. Some breeding groups had extra individuals added such that half the breeding groups consisted of five, six, or seven individuals. From these 26 pairs the target was to produce 110 offspring from each breeding group to ensure that each group made an equal contribution to the next generation. A target of 260 offspring was set for the 17 breeding groups. Approximately 6,900 fish were produced at the University of Canberra, 2,700 in the first round of breeding for Deception Creek and 4,200 in the second round of breeding for Puzzle Creek. Eggs were collected on synthetic wool mops placed into breeding tanks. After 2 days of spawning, the mops were transferred to small fish tanks (40 × 20 × 20 cm) and the juvenile fish were raised for approximately 2 months before being transferred to larger tanks (91 × 35 × 45 cm). Breeding and rearing tanks had painted sides and bottom and a sponge filter. Larvae were started on a diet of live vinegar eels (*Turbatrix aceti*), and as they grew larger moved onto a diet of juvenile brine shrimp (*Artemia* sp.) over the course of about a week, together with commercial flake food.

Once large enough for transport, the fish were air-freighted to James Cook University (JCU) Townsville and distributed evenly into 10 outdoor rearing ponds (108 cm in diameter and 36 cm deep, 330 L) to grow out. At JCU, the fish were fed with commercially available flake food three times a day and a mixture of frozen brine shrimp and blood worms (Chironomidae) once a day. All rearing ponds contained several large river stones and plastic mesh 50 × 100 cm with holes of 2.5 cm in diameter, which was contorted into different shapes and added to provide cover. This was to encourage natural behaviours such as using cover to escape threats, establishing and holding territories, and foraging, which have previously been found to result in improved survival rates (Brown, Davidson & Laland, 2003; Roberts et al., 2014). Although there were differences in the shape and size of the rocks, all the ponds were arranged in a similar pattern.

2.2 | Predator training

Release sites in Deception Creek were known to contain a potential predator, the spangled perch. To test the impact of predator training, half of the rearing ponds were exposed to an adult spangled perch of approximately 15 cm in length placed in a 25 × 25 cm 'mesh box' made from plastic 2.5-cm mesh within the outdoor pond. RRR were able to swim freely in and out of the mesh box. In addition to providing the predator, a cutaneous alarm cue was also provided, which is often released when the skin of a fish is damaged and can be used in associative learning (Brown, 2003b; Brown & Chivers, 2007; Abudayah & Mathis, 2016). To obtain this alarm cue one RRR was euthanized (with an overdose of clove oil) per week of training, crushed up, mixed with water, and sieved to remove larger fragments.

This solution was then frozen in an ice-cube tray and one cube was added at the same time as the spangled perch in the hope that juvenile RRR would associate the olfactory cue of dead or injured conspecifics with the stimulus of a spangled perch. The spangled perch was left in the rearing pond for 15 min per day for 7 days immediately before the fish were released into the wild.

2.3 | Release sites

Deception Creek, which flows into Running River just below the lowermost gorge, and Puzzle Creek, which flows into Running River just above the uppermost gorge, were identified as the best potential translocation sites (Figure 1). Both creeks contained barriers to the upstream dispersal of rainbowfish (Figure 1) and already had resident fish fauna, meaning that the potential impacts on invertebrates and frogs of introducing a new fish species was minimal. Throughout most of the year Deception Creek consists of disconnected pools without flow, whereas Puzzle Creek flows for most of the year but with reduced/disconnected pools during periods of low rainfall. Purple spotted gudgeon was found in both creeks, whereas spangled perch was found throughout Deception Creek and in reaches below the release sites in Puzzle Creek. Although both species have the potential to prey upon small fishes, spangled perch grows to a much larger size than purple spotted gudgeon and are more active hunters (Pusey, Kennard & Arthington, 2004). Therefore, as the predation pressure on small fishes in Deception Creek was likely to be higher than that in Puzzle Creek, releases into Deception Creek were used to assess the effect of predator training on translocation success.

In an attempt to isolate the effects of predator training, the release sites within Deception Creek were paired based on similarities between habitat variables, with one site randomly selected to receive trained fish and with the other site receiving untrained fish. Puzzle Creek release sites were also assessed, but owing to the lower number of accessible pools, habitat assessments were only used to identify suitable release sites. The habitat variables examined were pool length, average pool width, substrate composition, average depth, deepest point, and riparian cover. Pool length was measured from the uppermost water edge to the farthest downstream water edge. Average pool width was calculated by taking three measurements at 25%, 50%, and 75% of the total length of the pool using a tape measure. A transect comprising five sample points was taken along each width measurement at 0% (+25 cm), 25%, 50%, 75%, and 100% (-25 cm) of the channel width. At each sample point, depth, substrate composition, macrophyte cover, and leaf litter were measured. Macrophyte cover, substrate, and leaf litter were all considered independent of one another. Macrophyte cover was defined as all emergent and submerged vegetation within the quadrat. Riparian cover was defined as the percentage of the bank covered by vegetation. Riparian cover was estimated by eye to the nearest 5%, whereas depth was measured using a metal ruler. All other variables were measured using a 50 × 50 cm quadrat.

Release pools were paired based on similar size, riparian cover, and substrate, in that order, with one pool randomly assigned to trained or untrained fish. As there were limits to the number of fish that could be produced, the 2,500 that were bred were divided into groups of 250 for release. This number was chosen to balance the number of release sites against the number of fish in each release.

2.4 | Release and monitoring

Ten releases of 250 fish were performed across 10 release sites in Deception Creek between 2 November 2016 and 13 January 2017. Releases were made in groups of 250 to provide five replicates of each treatment (trained and untrained), as grow-out facilities consisted of 10 ponds. At release, the fish were approximately 3 cm in total length, on average, but varied from approximately 2 to 5 cm. Deception Creek releases occurred once every week or so; however, there was no assigned order for which releases happened when, owing to logistical constraints regarding predator avoidance training. Fish were transported from rearing ponds at JCU to their release sites in 20-L plastic buckets. Buckets were filled to one-third full and water was dosed with sea salt at 2.6 g L^{-1} and API Stress Coat® (Mars Fishcare, Inc., Chalfont, PA, USA), dosed at 0.8 ml L^{-1} . Fish were delivered to their release site on the same day as collection from the rearing ponds in all but one case, which was hampered by heavy rainfall. In this instance, fish were held in buckets for 2 days with a daily water change, before delivery to their release site. Fish were held instream at the release site overnight in a holding net with dimensions of $1 \times 1 \times 1 \text{ m}$ made from shade cloth and polyvinyl chloride (PVC) pipe. This allowed the fish to acclimatize to water conditions without any predation pressure. The following day the fish were released into the pool by gently up-ending the holding net.

After release, snorkel surveys were used to estimate the abundance of spangled perch and RRR in each pool. Snorkel surveys were chosen as the survey method needed to be non-destructive and non-intrusive. A small pilot study was conducted early on, comparing the detection rates among snorkel surveys, bait traps, and baited remote underwater video; however, the latter two methods did not detect a single RRR (K. Moy, unpublished data). Owing to logistical constraints, surveys occurred somewhat opportunistically. However, at least one survey was undertaken in the first week following release and this was often followed by other surveys up to 56 days after release. Forty-one surveys across five untrained and two trained release sites were made between 2 November 2016 and 5 January 2017. A large rainfall event (over 200 ml across 4 days at the nearest rainfall gauge) occurred in early January 2017, which caused flooding and restored flow to the channel, reconnecting the release pools before the predator training experiment in Deception Creek could be completed. This prevented any survey data being collected for the final three releases, which were all of trained fish. Snorkel surveys consisted of three passes: along the left bank, then the right bank, and

with a final pass down the centre of the pool. The researcher kept a steady pace to prevent any double counting of fish, and on a waterproof notepad recorded a tally of the total number seen as well as the maximum seen at any one time, with a separate count for larvae. Spangled perch were also recorded in this way to estimate predator density. Follow-up surveys were undertaken for all sites in Deception Creek in May and October 2017.

After the first field season, the extent of fish occurrence throughout each drainage was mapped by walking along the creek, upstream and downstream from the uppermost and lowermost pools, respectively, and stopping at each pool encountered for 5 min to observe the presence or absence of rainbowfish. If no rainbowfish were observed within 5 min, the researcher moved to a different region of the pool and continued to observe for a further 5 min. If no rainbowfish were observed, the next pool downstream or upstream was also checked. This was repeated until three pools in a row were found without rainbowfish. This was carried out for Deception Creek in May and October 2017 and in April 2018. An attempt was made to map the extent of RRR in Deception Creek after the large rainfall event in early January 2017, following the same protocol above, but was hampered by low visibility owing to the increased turbidity.

Four releases, each consisting of 375 untrained fish, were made into four sites across Puzzle Creek in May 2017 in the same manner as those made into Deception Creek. Although the fish released into Puzzle Creek were the same size as those released into Deception Creek, only 1,500 of the originally intended 4,000 fish were released because of attrition in the rearing ponds. Owing to funding and weather constraints on fieldwork, no monitoring was undertaken in the weeks immediately after release for the Puzzle Creek releases. The planned monitoring of Puzzle Creek in October 2017 was prevented by a large rainfall event, but a survey of all release sites following the same protocol described above took place in May 2018. Distribution mapping for Puzzle Creek took place in May 2018 following the same protocol used for Deception Creek. Research was conducted under the University of Canberra Animal Ethics Committee approval CEAE 16-03.

2.5 | Analysis

Two-sample Student's *t*-tests were used to test for differences in abundance in Deception Creek following release for trained versus untrained fish sites, paired by habitat variables, whereas an independent-samples Student's *t*-test was used to look for differences in density between releases made before and after flooding. For observations not made in the month immediately after release, measures of abundance from the surveys were converted into measures of density by dividing the abundance by the length of the pool. Two-sample Student's *t*-tests were used to determine differences in density between trained and untrained release sites within Deception Creek from data collected during May and October (approximately 6 and 8 months from release).

3 | RESULTS

3.1 | Crowdfunding

A total of AU\$26,465 was raised from donations made by individuals (AU\$4,435), companies (AU\$1,150), and aquarium clubs (AU\$20,880), with donations received from Australia, USA, Canada, Switzerland, and Germany. The largest donation was AU\$10,000 from the Aquarium Society of Victoria. Most donations from aquarium clubs were solicited through personal contacts. Without these funds the project would have been impossible and RRR would be close to extinction. Crowdfunding covered all of the DNA sequencing costs, fish food, and live fish shipping, which cost approximately AU\$12,000 in total. The bulk of the remaining funds were used over subsequent years to continue monitoring the wild and translocated populations, including further genetic monitoring.

3.2 | Habitat

In October 2016, release sites in Deception Creek varied between 100 and 280 m in length and between 8 and 14 m in width. The average depth varied between 42 and 113 cm, whereas the deepest points ranged from 1.65 to 3.00 m. Riparian cover ranged from 60% to 99%. Substrate was dominated by sand (45%–95%), followed by boulder (0%–26%), bedrock (0%–24%), and cobble (0%–17%). On average, aquatic plants (macrophytes and charophytes) covered approximately 40% of the substrate, whereas leaf litter covered approximately 25% of the substrate. Release sites within Puzzle Creek were between 150 and 265 m in length and between 9.9 and 22.4 m in width, with the average depth ranging between 84 and 125 cm, and with the deepest points ranging from 1.70 to 2.75 m. Riparian cover varied between 95% and 80%, whereas the average substrate was dominated by sand (40%–60%), followed by bedrock (3%–43%), cobble (7%–32%), and boulder (2%–7%). On average, aquatic macrophytes and charophytes covered 20% of the substrate, whereas leaf litter covered 20% of the substrate.

3.3 | Predator effects

There was no significant difference in abundance or density of adult fish between trained and untrained release sites at any point after release (Table 1). Of the seven releases in Deception Creek before flooding, fish failed to become established at only one site following the release of untrained fish. This site was surveyed five times from 2–31 days after release without a single RRR observed, and was similar to other sites in every way. At the remaining sites the abundance of released fish appeared to decline continuously over the 56-day monitoring period for both treatments at sites where samples were collected for more than 2 weeks following release (Figure 2). However, linear regression analysis did not provide statistical support for this decline ($t = 0.27$, $P = 0.788$), although this could have been the result of the low detection power caused by small sample sizes and variation in detectability. Increasing numbers of detected fish at some sites over the first few days after release (Figure 2) were probably the result of fish becoming more familiar with their new environment.

Regression analysis found no significant link between predator density and RRR abundance or density for any survey season (Table 2). This was the case even when the analysis was broken up into different size classes for both RRR and spangled perch. Although these results were not statistically significant, there was a positive correlation between adult RRR density and the density of all spangled perch (Appendix S1).

Fry of RRR were detected within the first field season at four sites (two trained and two untrained) 30–40 days after release. In May 2017, both juveniles and adults that were too small to have been the released fish were detected at all sites. When the total density of RRR – including fry and juveniles – was compared, untrained release sites had significantly higher densities than trained release sites at 6 months after release, but at no other time (Table 1). No significant difference in RRR density was found between releases that took place before or after the flooding that occurred between the May ($t = -1.91$, $P = 0.09$) and October ($t = 0.557$, $P = 0.59$) surveys.

TABLE 1 Statistical output comparing trained and untrained releases of fish. A Welch's *t*-test (*W*) compared the total observed abundance at 2–3 weeks from release, whereas a paired Student's *t*-test (*P*) compared the density of adults at 6 and 11 months from release. The standard error (SE) was calculated from 11 abundance observations between two sites that all fell within 4 days of one another, converted to a percentage and then applied to all samples.

	<i>t</i> -test	Trained ± SE	Untrained ± SE	<i>T</i>	<i>P</i>	<i>df</i>
Adults						
2–3 weeks	<i>W</i>	85.7 ± 18.85	38.25 ± 8.42	−1.60	0.260	1.85
6 months	<i>P</i>	1.3 ± 0.30	1.86 ± 0.41	−2.14	0.100	4
11 months	<i>P</i>	2.0 ± 0.45	1.58 ± 0.35	−0.65	0.554	4
Juveniles						
6 months	<i>P</i>	0.2 ± 0.04	0.36 ± 0.07	−1.22	0.291	4
11 months	<i>P</i>	1.3 ± 0.28	1.06 ± 0.21	−0.67	0.542	4
All rainbowfish						
6 months	<i>P</i>	1.5 ± 0.44	2.22 ± 0.49	−2.88	0.045	4
11 months	<i>P</i>	3.1 ± 0.62	2.97 ± 0.59	0.11	0.920	4

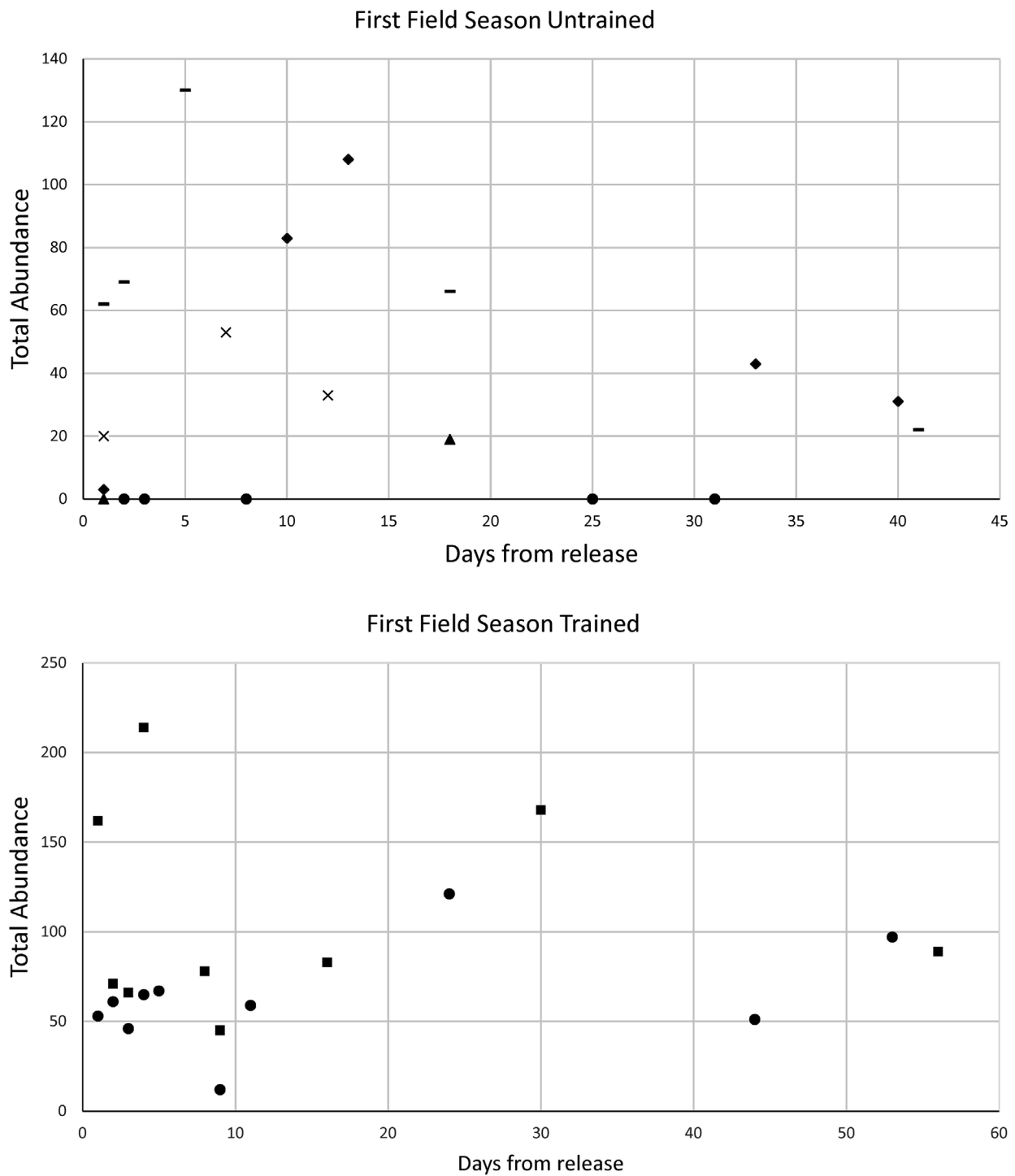


FIGURE 2 Abundance of released Running River rainbowfish over time during the first field season in Deception Creek for trained and untrained fish. Different markers represent different release sites. Note, the number of released fish cannot increase, as fish were only released once into each site.

Unfortunately, only one survey of Puzzle Creek was made after release, as all other attempts were prevented by heavy rain and flooding. Flooding occurred between the release and the survey, and as a result the data from the Puzzle Creek survey were not analysed.

Anecdotal observations in Deception Creek made in the hours and days immediately after release suggest that there may have been some behavioural differences between trained and untrained fish. In both pre-flood releases, the trained fish shoaled together close to the

point of release and found a shallow, sandy area out of the reach of larger spangled perch and remained there for around 6 days before dispersing more widely. In contrast, untrained fish were often observed swimming near the surface in open water and swimming towards the spangled perch, which were trying to eat them, before eventually finding shallow areas in which to hide.

3.4 | Dispersal

When flooding occurred in Deception Creek the RRR moved between release sites, invalidating any comparisons between treatment pools. Ten days after flooding in Deception Creek, one individual RRR was recorded in an ephemeral gully stream 660 m upstream from Deception Creek and approximately 24 m higher in elevation than the nearest release site. The movements of fish from their uppermost and lowermost release sites in both systems are summarized in Table 3. The population in Deception Creek spread upstream and downstream much faster than the fish in Puzzle Creek (Table 3). In 1 year, RRR from Puzzle Creek dispersed a total of 460 m upstream, 200 m less than the distance covered by a fish from Deception Creek in 10 days. In Deception Creek there was a large increase in the distance spread downstream between October 2017 and April 2018 (Table 3). The maximum distance of spread downstream in Deception Creek in April 2018 could not be determined because of time constraints and limited access to that portion of the creek.

4 | DISCUSSION

4.1 | Summary

This study documents efforts to conserve a Critically Endangered species threatened by the establishment of an alien species. This was

TABLE 2 Statistical output from linear regression analysis testing predator density as a predictor of rainbowfish abundance in the first month, and density at 6 and 11 months after release.

	<i>T</i>	<i>P</i>	<i>R</i>	<i>df</i>
2–3 weeks	0.527	0.621	−0.137	5
6 months	−0.014	0.989	−0.125	8
11 months	1.545	0.161	0.133	8

TABLE 3 Upstream and downstream movements of Running River rainbowfish from their release sites in Deception and Puzzle creeks over time.

	Time since release	Distance (elevation)	
		Upstream	Downstream
Deception Creek May 2017	6 months	1.9 km (31 m)	1.3 km (46 m)
Deception Creek October 2017	11 months	2.4 km (39 m)	2.7 km (62 m)
Deception Creek April 2018	17 months	2.5 km (41 m)	>6.3 km (>171 m)
Puzzle Creek May 2018	12 months	0.46 km (9 m)	1.33 km (30 m)

achieved by translocating captive-bred offspring to two unoccupied creeks isolated by large waterfalls. The conservation actions to save the RRR were an outstanding success, given that they persist in the wild adjacent to their native range, and the research and monitoring accompanying the translocation releases aims to draw lessons on techniques and habitat selection for similar future projects. Additionally, it provides insights into the rate that rainbowfish may spread through a system.

4.2 | Predator training

Although the small sample sizes in this experiment meant that only major differences could be detected, the data presented here do not support the hypothesis that predator training (exposure to predators prior to release) or predation pressure influenced the introduction success in RRR. Although the only unsuccessful release was of untrained fish, all other releases of untrained fish were successful, suggesting that predator-naive fish are still capable of becoming established in the right circumstances. As rainbowfish are known to use social learning (Brown & Warburton, 1999b), and as experienced fish from other releases were observed at post-flood release sites, it is likely that post-flood releases were less affected by predation encounters than pre-flood releases. Introductions into Puzzle Creek were made during a high-flow event and yet still established a sustaining population, so it is likely that the post-flood releases in Deception Creek survived to reproduction. Few released fish, if any, were present at release sites 6 months later, as most fish observed were smaller than the individuals released, and thus it was likely that most of the fish observed were spawned in the wild. Therefore, owing to the high fecundity of rainbowfishes (Milton & Arthington, 1984; Pusey et al., 2001), differences in rainbowfish density would not be expected at 6 or 11 months after the releases. As the rainfall, flow regime, habitat, vegetation, and resident fish biota of Puzzle Creek were different from that of Deception Creek, and Puzzle Creek was only surveyed once, the conclusions that can be drawn from this translocation are limited. It can, however, be said that predation and competition with purple spotted gudgeon and flooding during introduction did not prevent RRR from becoming established.

Although unquantified, the anecdotal observations made in the hours and days immediately after the Deception Creek releases followed the findings of Brown & Warburton (1999a), where naive rainbowfish were less able to evade danger than experienced ones.

One reason that predation may not have had a significant impact is that neither spangled perch nor purple spotted gudgeon are primarily piscivorous (Pusey, Kennard & Arthington, 2004). The presence of a more specialized piscivore, such as the mouth almighty (*Glossamia aprion*), might have produced a different outcome. The mouth almighty has been implicated in the extirpation of the Lake Eacham rainbowfish (*M. eachamensis*) from Lake Eacham (Barlow, Hogan & Rodger, 1987), and it is not unreasonable that a similarly proficient piscivore could have adverse impacts on an introduction of small-bodied fish if they did not possess the ability to recognize or escape predators (Brown & Warburton, 1997).

4.3 | Translocation success

The RRR releases were an uncommon success for Australian freshwater fish conservation translocations, which could be explained by several factors that were likely to be working in unison. First, eggs were observed within the overnight instream holding pen at some sites before the fish were released the following morning. The use of well-conditioned, sexually mature fish under conditions favourable for spawning allows them to do so on the first day, which has obvious benefits when trying to establish a new population. Second, the fish were given a soft release (with a gradual transition from captivity to nature) to allow them to adjust to the water parameters of the receiving site and recover from handling or transport stress. It has been known for some time that handling and transport not only causes stress and in turn reduced survival rates in fishes, but that the effects can linger for some time afterwards (Hattingh, Le Roux Fourie & van Vuren, 1975; Iversen, Finstad & Nilssen, 1998). However, the approach is not commonly used in fish releases and may therefore be one area in which future fish releases could improve. This soft-release approach had the added effect of allowing fish to reproduce in a protected area for a short time.

4.4 | Dispersal

Although there is a paucity of information regarding the movements of Australian small-bodied freshwater fishes, studies on ephemeral waterholes (Kerezsy et al., 2013) and genetics (Unmack, Allen & Johnson, 2013) suggest that some of these species are capable of dispersing great distances. The study of dispersal in small-bodied fishes has often been hampered by their size and the consequent limitations in employing individually tagged fish (Allan et al., 2018). However, these releases in a stream of low turbidity, where snorkelling could be used as a monitoring method, provided a unique opportunity to understand the rate at which rainbowfishes may spread throughout a previously unoccupied waterway. Puzzle Creek flows more frequently than Deception Creek, suggesting that expansion throughout Puzzle Creek could occur much faster. Although fewer fish were stocked into Puzzle Creek, the fecundity of the species should have counteracted any effect that this may have

had on dispersal, meaning it was reasonable to assume that RRR would spread through Puzzle Creek at a similar if not faster rate. Contrary to what might have been expected, the RRR dispersed throughout Deception Creek faster than Puzzle Creek.

One possible explanation is that although the same number of fish per pool were released into Puzzle Creek, these pools were much larger and better connected than those in Deception Creek, resulting in lower densities of adult fish. This may have been exacerbated by flooding at the time of release, which may have encouraged dispersal. Some locations that fish dispersed to will not provide long-term habitat during dry periods, and it is almost certain that many fish died after dispersal in Deception Creek, as many individuals were observed occupying more temporary habitats (e.g. the individuals observed within the ephemeral gully). In Deception Creek, however, opportunities to disperse were less frequent and were initially limited, restricting released fish to their release sites where they increased in population size, thereby increasing the success of subsequent dispersal. The site fidelity of translocated individuals is consistently lower than that of wild individuals across most faunal groups (Clarke & Schedvin, 1997; Tuberville et al., 2005), including fish (Ebner & Thiem, 2009). Immediate dispersal from the point of release may increase the likelihood of translocation failure, as individuals may disperse to suboptimal habitats, encounter predators in unfamiliar environments, become so thinly distributed that Allee effects increase, and so forth. In some instances, 'penning', whereby translocated organisms are kept in pens at the release site for several days or weeks before being allowed to roam free, has been an effective method of increasing site fidelity and the overall success of establishment (Tuberville et al., 2005). It is possible that during periods of low flow, disconnected pools acted in a similar fashion, forcing fish to develop some site fidelity with their new habitat and allowing them to increase in number, thereby increasing the number of fish that dispersed when it became possible to do so.

4.5 | Lessons learned

Translocations are becoming an increasingly important conservation tool the world over, especially for small-bodied fishes. The findings of this study are discussed in the context of Australia; however, the issues faced here are likely to be relevant globally. Despite its importance in formulating effective conservation translocation plans, there are few studies incorporating robust follow-up monitoring on Australian native fish releases (Lintermans, 2013b) or survival in the weeks immediately after release. A recent review of threatened species monitoring in Australia found significant deficiencies for all vertebrate faunal groups (Scheele et al., 2019), as well as for freshwater fishes specifically (Lintermans & Robinson, 2018). In the present study, monitoring showed that the failed release had failed within 2 days of the release. External factors and small sample sizes that are typical of conservation translocations make it difficult to assess adequately the effect of predator training on post-release survival. Our anecdotal observations of different behaviours

immediately after release suggest that this would be a fruitful area for further investigation (Berger-Tal, Blumstein & Swaisgood, 2020). Given the length of time required to examine long-term survival, we recommend that future studies focus on behavioural deficiencies occurring in the immediate period after release. Owing to its low cost and support from laboratory-based experiments (Vilhunen, 2006; Hutchison et al., 2012), we recommend the continued implementation of predator training in release programmes.

Anecdotal observations from successful releases indicated that the captive-reared fish introduced to Deception Creek gradually decreased in abundance over time. Natural processes such as predation and finding suitable resources, combined with the behavioural deficiencies of captive-reared fish, made such declines likely. However, upon release the fish were able to reproduce during periods of low flow and elevated temperatures, which are ideal spawning conditions for the other rainbowfish species in northern Queensland (Pusey et al., 2001), allowing the population to grow quickly and overcome initial declines. This suggests that the time of year that a release takes place may play an important role in determining whether or not it is successful. Owing to constraints on funding and time, it was not possible to obtain detailed information on the initial population growth for fish in Puzzle Creek in the first months after release. In contrast to Deception Creek, fish were released into Puzzle Creek at a time when conditions were not ideal for reproduction (e.g. with cooler temperatures, going into winter), and yet this still resulted in the successful establishment of a new population, highlighting that ideal conditions are not always necessary for establishment, at least in rainbowfishes.

Successful conservation introductions of Australian small-bodied freshwater fishes often take place in areas with no potential predators or competitors present, often to avoid non-native species that could prevent them from becoming established (Ayres, Nicol & Raadik, 2012; Chilcott et al., 2013). One of the main reasons for this is that predation or competition from alien species is often seen as a major cause of the decline of a species (Cadwallader, 1996; Lintermans, 2000; Morgan et al., 2003), and therefore conservation introductions are unlikely to succeed in locations where these alien predators or competitors are still present. Although negative interactions with alien species are the leading cause of decline in animal species globally (Clavero & García-Berthou, 2005; Bellard, Cassey & Blackburn, 2016; Allek et al., 2018), conservation introductions of RRR have shown that a complete lack of other species is not required. Studies on captive-reared fish have shown a rapid loss of behavioural traits, such as a loss of predator recognition (Alvarez & Nicieza, 2003) and a reduced competitive ability (Rhodes & Quinn, 1998), suggesting that the recovery or adequate conservation of a species will be detrimentally affected if the species is maintained away from all predators and competitors. We would suggest that when conservation introductions are required, and predation is not an overwhelming threat (e.g. when suitable shelter from predators is available), effort should be made to include a mix of predator-free and predator/competitor-present release sites or a staged release similar to that described by Robinson & Ward (2011).

Conservation translocations for RRR contrast with those of larger-bodied, long-lived species. Unlike releases for larger species (Minckley, 1995; Harig, Fausch & Young, 2000; Ebner, Johnston & Lintermans, 2009; Lintermans, 2013c), it was possible to determine whether or not these releases were successful over a much shorter time period, much like other small-bodied fish translocations (Minckley, 1995). This can probably be explained by two factors: first, the RRR were released into habitats free of the cause of decline (introgression/hybridization); and second, like most small-bodied species RRR reach maturity at a much younger age (e.g. 1 year in rainbowfish; Milton & Arthington, 1984), compared with large-bodied species (e.g. 3–4 years in the Macquarie perch, *Macquaria australasica*; Appleford, Anderson & Gooley, 1998). This means that released fish can reproduce in a relatively short period of time, so even if released fish exhibit behavioural deficiencies that inhibit long-term survival, wild-spawned fish free of these deficiencies will rapidly be present (Alvarez & Nicieza, 2003). However, it is also worth noting that a shorter lifespan poses an extra risk. Although it has already been noted that the conservation benefits of captive maintenance for a species may be limited (Philippart, 1995; Snyder et al., 1996; Araki, Cooper & Blouin, 2007; Attard et al., 2016), the short lifespans and generation times of most small-bodied species mean that the adverse effects of captive maintenance will take effect more quickly, and that stochastic events such as a reproductive failure can extinguish annual species rapidly.

This research has established two factors important for the continued management and conservation of small-bodied fish species: (i) that they may easily establish new populations when the dominant threat is removed and suitable habitat is available; and (ii) that conservation translocations for small-bodied fish species can be carried out on a moderately sized budget of AU\$10,000–20,000. Most small-bodied species are less likely to be intentionally translocated outside their natural range, compared with large-bodied species (Rahel, 2004; Hunt & Jones, 2017), and are more likely to enter a new area through other pathways such as bait-bucket translocations and stocking contamination (Ludwig & Leitch, 1996; Lintermans, 2004; Rahel, 2004). Given the number of widespread species complexes of small-bodied species in Australia (Page, Sharma & Hughes, 2004; Hammer et al., 2007; Raadik, 2014; Hammer et al., 2019a), the chance of an accidental translocation resulting in establishment, hybridization, and subsequent introgression is quite high. However, the ease with which populations may be established is also beneficial for the establishment of refuge populations used for conservation, assuming suitable refuge habitat is available.

Rainbowfish species with broad distributions (e.g. the eastern rainbowfish and the western rainbowfish) possess many traits that allow them to establish new populations quickly, and as a result the number of rainbowfish species threatened by translocation is likely to increase in the future. Many small-bodied species in Australia are likely to face the same challenges. To date, Australia can claim that it has experienced very few freshwater fish extinctions, with the limited examples being of undescribed taxa (Unmack, 2001; Faulks, Gilligan & Beheregaray, 2010), but this is unlikely to remain

the case in the future unless appropriate management measures are taken. To prevent future declines and extinctions, careful management and continued robust monitoring will be required. The establishment of conservation populations for small-bodied species should be more easily achieved, as they are easier to breed or translocate and have early maturity, but this effort requires a small but important investment of funds towards the conservation of smaller native fish.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

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