Threatened Species Recovery Hub

National **Environmental Science** Programme

The removal of non-native fish to help protect the Critically Endangered Spotted Tree Frog in year one of a six-year Management Trial

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Cover image: Spotted tree frog. Image: Matt West

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Summary

The Spotted Tree Frog, *Litoria spenceri*, has disappeared from 50% of historic sites, is rare at all remaining sites, and is expected to become extinct without intervention. Nationally, the Spotted Tree Frogs' conservation status is under review and expected to change to Critically Endangered from the current Endangered (EPBC Act) listing. Population declines are considered driven primarily by non-predatory native fish and disease caused by chytrid fungus. Currently, chytrid cannot be eliminated from the fast-flowing streams in which Spotted Tree Frogs breed. Non-native fish management is technically feasible and is a key action identified in the Spotted Tree Frog EPBC Conservation Advice.

A Non-Native Fish Management Trial was co-designed by a management committee (Spotted Tree Frog Threat Management Committee) with broad stakeholder representation from community groups (Recreational Fishers), Traditional Owners, land, water, and wildlife managers and scientists. The Trial objectives are to determine 1) if mechanical removal methods can reduce non-native fish at key frog sites, 2) if Spotted Tree Frog populations increase following non-native fish removal, and 3) if the resultant changes in the frog populations influence chytrid prevalence at sites. The Trial involves a 6-year before and after, control and impact (BACI) study design, mechanical removals of non-native fish (by angling and electrofishing), and mark-recapture surveys to evaluate changes in the frog and fish populations at two sites. The sites, located in the Big River Catchment of Victoria, were selected because they are crucial for the persistence of the Spotted Tree Frog, an existing fish barrier separates them, and the above the barrier (fish removal or "impact") site was determined to have low recreational fishing value. Additionally, the frog populations at the sites have been monitored (via mark-recapture surveys) since 1992 (29 years).

This report summarises an assessment of the "before" removal non-native fish population and non-native fish removals in the first year of the Trial. Fish population assessments were conducted in November 2020, April 2021, and May 2021 using a backpack electrofishing method and by tagging fish to establish a mark-recapture study along multiple 100m transects on both sides of the barrier. These fish transects overlapped the Spotted Tree Frog mark-recapture survey zones. Volunteer Anglers removed non-native fish above the barrier by hook and line over a weekend in early May, covering 6km of the stream above the barrier. Electrofishing teams removed non-native fish over one week along a 13km stream section above the barrier in mid-May.

Brown and Rainbow Trout were the only non-native fish species detected. While similar total numbers of trout were found in transects on each side of the barrier, Rainbow Trout were the most prevalent non-native fish species above the barrier, and Brown Trout were the most prevalent species below the barrier. Fish capture probabilities, determined via simple mark-recapture analysis, were used to approximate trout abundance from single-pass electrofishing counts. Around 7 – 11 Brown Trout and 34 – 51 Rainbow Trout/100m may occur above the barrier, and 33 – 49 Brown Trout and 15 – 23 Rainbow Trout/100m may occur below the barrier.

In the first year of the Trial, the combined efforts of Volunteer Anglers and Electrofishing teams resulted in the removal of 3086 trout from the stream above the barrier, including 2306 Rainbow Trout and 780 Brown Trout. More fish were removed by electrofishing compared to angling. However, the volunteer angler weekend was a crucial engagement activity that allowed the recreational fishing community representatives to evaluate the fishery and learn about the challenges of protecting the Spotted Tree Frog. The Management Committee now recommends that anglers and conservation practitioners focus efforts on electrofishing removals in the second year of the Trial. Work in the second year should 1) evaluate the effectiveness of the mechanical fish removal methods, 2) strive to remove additional non-native fish, and 3) investigate initial Spotted Tree Frog population responses to first-year fish removals.

Background

The Spotted Tree Frog, *Litoria spenceri*, is an obligate stream breeder that has disappeared from 50% of historic sites, is rare at all remaining sites, and is expected to become extinct without intervention (West 2015). The species is listed as Critically Endangered in New South Wales under the Threatened Species Conservation Act 1995 and in Victorian under the Flora and Fauna Guarantee Act 1988. Nationally, the Spotted Tree Frogs' conservation status is under review and expected to change to Critically Endangered from the current Endangered listing on Schedule 1 of the Environment Protection and Biodiversity Conservation Act 1999 (Cwlth) (EPBC Act).

Non-native predatory fish and disease caused by chytrid fungus (*Batrachochytrium dendrobatidis*) are the primary threats to the persistence of the Spotted Tree Frog. Non-native Brown (*Salmo trutta*) and Rainbow Trout (*Oncorhynchus mykiss*) eat Spotted Tree Frogs tadpoles (Gillespie 2001). In contrast, Spotted Tree Frog tadpoles appear to be unpalatable for native fish like Two-spined (*Gadopsis bispinosus*) and River Blackfish (*G. marmoratus*) (Gillespie 2001). Chytrid causes disease (chytridiomycosis) and reduces the survival of adult frogs (Gillespie *et al.* 2015; West 2015). Under some conditions, Spotted Tree Frog populations may be able to cope with one of these threats, but they cannot manage when both threats are present and when environmental conditions are optimal for chytrid and trout (West *et al.* 2020). Chytrid and non-native predatory fish species are present at all remaining Spotted Tree Frog sites (West & Johnson 2020). Chytrid cannot yet be eliminated from the fast-flowing streams in which Spotted Tree Frogs breed, whereas non-native fish removal is technically feasible (West *et al.* 2020).

Brown and Rainbow Trout are widely regarded as important recreational fish species and are highly valued by recreational fishing communities in south-eastern Australia (Jackson *et al.* 2004; Victorian Fisheries Authority 2020). Both species were introduced to Australia in the second half of the nineteenth century (Brown Trout arriving from Europe and Rainbow Trout arriving from North America) (Crowl, Townsend & McIntosh 1992). Self-supporting Brown and Rainbow Trout populations now occur at all known Spotted Tree Frog sites, some stockings of trout still occur (Victorian Fishers Authority 2021), and the majority of Spotted Tree Frog sites are also frequently used by recreational anglers (Hawkins *et al.* 2019). A Recreational Fisher's Advisory Group was established in 2017 to help the Spotted Tree Frog Recovery Team identify technically feasible management strategies to protect the Spotted Tree Frog that are acceptable to the broader community. One of the first steps undertaken by the Advisory Group was to identify and map sites that are both of high value for Spotted Tree Frogs and low value for recreational fishing. In May 2019, the Group was expanded to include Traditional Owners, land, water, and wildlife managers and scientists. This newly formed Spotted Tree Frog Threat Management Committee currently includes representation from the Australian Trout Foundation, Arthur Rylah Institute for Research, Dept. of Environment, Land, Water, and Planning, Goulburn Broken Catchment Management Authority, Murrindindi Shire Council, Native Fish Australia (Vic), Parks Victoria, Taungurung Land and Waters Council, Victorian Fisheries Authority, Victorian Recreational Fishing Peak Body, University of Melbourne and Zoos Victoria. The Committee developed a Non-native Fish Management Trial to evaluate the influence of non-native fish on Spotted Tree Frog populations. The Committee also agreed on a site to conduct the Trial that was important for the Spotted Tree Frog but of relative low value for the recreational fishing community.

The Non-Native Fish Management Trial outlines a 6-year "before and after-control and impact (BACI)" study involving the mechanical reduction of non-native fish to alleviate and evaluate predation pressure on Spotted Tree Frogs. The Trial builds on a 29-year mark-recapture study of Spotted Tree Frog populations in the Big River catchment of Victoria (Gillespie 2010; Gillespie & West 2012; West 2015; West *et al.* 2020). A concrete fish barrier (Fig. 1) had been installed between two Spotted Tree Frog monitoring sites in the catchment in the early 2000s to undertake a similar fish removal experiment. However, due to community concern about the proposed use of a piscicide, fish were not removed at the time. Stakeholders, co-designing the new 2021 Trial, agreed that recreational fishers (Volunteer Anglers) and conservation practitioners (Electrofishing Teams) would collaborate to reduce non-native fish above the barrier using angling and electrofishing methods. During the Trial changes in fish and frog populations will be assessed at a "control" site below and an "impact" site above the barrier "before" and "after" fish removals from the above barrier site. The objectives of the 6-year Non-Native Fish Management Trial (2021 -2026) are to:

1. Determine the feasibility and effectiveness of mechanical methods at reducing population densities of non-native fish.
2. Determine if there is a measurable increase in the juvenile and adult Spotted Tree Frog population size following a reduction of non-native fish.
3. Determine if there is a measurable change in the prevalence of chytrid-infected Spotted Tree Frogs following the reduction of non-native fish.

The Management Trial addresses long-standing key management actions identified in the expired National Spotted Tree Frog Recovery Plan (1998-2002), the Spotted Tree Frog FFG Action Statement, and the Spotted Tree Frog EPBC Conservation Advice (2017). The project also aligns with the objectives of the Victorian Waterway Management Strategy, the Goulburn Broken CMA Regional Catchment Strategy, the Goulburn Broken CMA Waterway Strategy, Murray–Darling Basin Native Fish Recovery Strategy, the Protecting Victoria's Environment – Biodiversity 2037 Plan, and the Australian Government's Threat Abatement Plan for infection of amphibians with chytrid fungus resulting in chytridiomycosis (2016).

The "before removal" fish population assessments commenced in November 2020, and the first-year fish removals began in May 2021. Work in the first year sets the stage for addressing all three Management Trial objectives, and the results of the fish surveys and removals are discussed in this report. In addition, an assessment of the changes in the Spotted Tree Frog populations during the 29-year mark-recapture study prior to fish removals is currently is currently being prepared as a manuscript for peer review and publication.

Figure 1. *The concrete barrier to prevent upstream fish movement within the Big River catchment, Victoria; (top) looking at the face of the barrier, and (bottom) from the barrier looking downstream. Images: Matt West.*

Aims

The project aims in the first year were:

1. To determine which fish species are present above and below the barrier before commencing non-native fish removals.
2. To establish a mark-recapture program that can evaluate changes in the abundance of fish above and below the barrier during the non-native fish Management Trial.
3. To reduce the number of non-native fish above the barrier in the first year of the Management Trial using mechanical (angling and electrofishing) methods.

Methods

Fish Population Assessments "Before" Removals:

Fish mark-recapture surveys commenced on the 4 - 6 November 2020 (November 2020 Assessment) and were repeated from 12 – 14 April 2021 (April 2021 Assessment) and 17 – 19 May 2021 (May Assessment Period). Single-pass surveys were undertaken by two operators using Smith and Root electrofishing backpack units and handheld dipnets. During the November 2020 Assessment, two electrofishing teams conducted the surveys along a continuous 1.2km section above and 1.2km below the fish barrier (total surveyed distance = 2.5km). These transects above and below the barrier entirely overlap the 1km transects assessed during Spotted Tree Frog mark-recapture surveys. During the April 2021 Assessment, two teams conducted surveys along the same stream sections but covered 850m above and 750m below the barrier (total surveyed distance = 1.6km). During the May 2021 Assessment, two teams conducted surveys along the same stream sections 1.2km above and 400m below the barrier (total surveyed distance 1.6km). During each assessment period, the electrofishing teams worked in an upstream direction to catch fish along 100m transect intervals (two transects were 150m in April). The electrofishing effort in seconds was recorded for each transect interval. All native and non-native fish captured within each transect were processed by a second team who checked the fish for existing tags, identified the species, measured body length, and tagged untagged fish. Fish were tagged according to the following rules:

1. All fish $\leq$ 85 mm were released without a tag;
2. All fish > 85mm were implanted with a small (8mm) PIT tag.
3. Fish >160mm length were implanted with a small (12 mm) PIT tag, and most were also tagged with a small (65mm long) T-bar tag. The T-bar tags included a unique code and phone number so that recreational anglers could report tagged fish if found.

During the November 2020 and April 2021 Assessments, all fish were released at the capture site once processed. However, during the May 2021 Assessment, native fish were released at the capture site, but non-native fish were only released if caught at the below barrier site. All non-native fish caught above the barrier in May 2021 were removed.

Angler Non-native Fish Removals in Year 1:

Twelve volunteer anglers on 1-2 May 2021 used hook and line methods to remove non-native fish above the barrier. In most cases, this involved fly-fishing with barbless hooks, but two anglers used rods and barbed-fishing lures. The anglers selected stream sections above the barrier, and generally, these sections were close to tracks and roads. The number and species of fish removed by each angler were recorded. Captured fish were weighed, measured, and checked for existing tags. When feasible, fish were translocated in an aerated fish transport tank to another area approximately 30km from the management sites. The release site was also below a dam wall to prevent fish from returning to the sites. Fish translocations were only feasible when captured within approximately 100m of a track or road and when anglers (and supporting staff) could safely carry the fish to the transportation tank. Other fish were humanely killed.

Electrofishing Non-native Fish Removals in Year 1:

During 17 – 21 May, two electrofishing teams used Smith and Root backpack electrofishing units to remove non-native fish. Single-pass electrofishing removals were conducted along 100m transects above the barrier (for 1.3km) and then in 100-500m intervals beyond this point, covering a total of 13km in the 5-day period. Electrofishing removals were conducted at three 100m transects on two consecutive days; other sites were only subject to one removal. Captured fish were processed by a team on the bank that recorded the number of each fish species, fish body weight and length, and existing tag numbers. The electrofishing effort in seconds was recorded in each transect interval. When feasible, captured fish were translocated to an area 30km's away (at the same site as during the Angler Non-native Fish Removals). Other fish were humanely killed.

Trout Mark-Recapture Analysis

A relatively simple Bayesian formulation of a spatially explicit mark-recapture model was used to estimate the probability of trout apparent annual survival, dispersal probabilities between transects, and capture probability. The model was adapted from a Jolly-Seber analysis described by Yackulic *et al.* (2020) to reflect the sampling approach used in the "before" Management Trial November 2020 – May 2021 surveys. Data were combined for all trout captured to approximate trout capture probabilities in the before-removal period. Separate models for each species will be developed for future years of the Management Trial. The model was fitted to the data using Markov chain Monte Carlo (MCMC) sampling in JAGS version 4.2.0 (Plummer 2015) and called from program R using the JagsUI package (Kellner 2017). Parameter estimates and their 95% credible intervals (95% CIs) were drawn from 10,000 MCMC samples after a burn-in of 5,000 samples and an initial adaptation of 100 iterations. Model convergence was assessed over the three Markov chains using the Gelman-Rubin statistic with an R-hat <1.01 considered an acceptable convergence threshold.

Results

Fish Assessment and Removal Conditions:

Streamflow during the Assessment and Removal Periods varied. In November 2020, stream flows were above average and were ranked (via an ARI traffic light system) as "Amber conditions", meaning that the capture probabilities of fish would be reduced due to the swift current and relatively high water turbidity. During the April and May 2021 Assessments and May 2021 Removal periods, streams flows were ranked as "Green conditions", meaning optimal survey conditions for species detection.

Survey Effort:

November 2020: On average, 574 electrofishing seconds were spent at each 100m transect. Average time spent electrofishing was reasonably consistent both above and below the barrier (average effort above barrier: 589.2 s/100m transect, range: 375 – 995 s/100m section; average effort below barrier: 559 s/100m transect, range: 415 – 677 s/100m section) (Fig. 1). **April 2021:** On average, 686 electrofishing seconds were spent at each 100m transect; with average effort above barrier: 659 s/100m section, range: 480 – 961 s/100m transect; and an average effort below barrier: 713 s/100m section, range:530.7 – 961 s/100m transect. **May 2021:** On average 671 electrofishing seconds were spent at each 100m transect; with average effort above barrier: 595.2 s/100m transect, range: 473 – 950 s/100m section; average effort below barrier was greater: 747.5 s/100m transect, range:654 – 843 s/100m section.

Fish Assessment Results:

Fish numbers in November 2020: A total of 335 fish were observed, including 191 Two-spined Blackfish (*Gadopsis bispinosus*), 57 Brown Trout, 56 Rainbow Trout, 30 additional trout (species not determined as they were not captured), and one Galaxiid (*Galaxias sp.*). Of those observed, 60% (n=201) were captured and tagged (Table 1). More blackfish were recorded above than below the barrier (8.8 and 7.4 Blackfish/100m, respectively). While both trout species were recorded on either side of the fish barrier, the predominant trout species differed. Rainbow Trout (RT) were the predominant non-native species captured above the barrier (mean of 1.7 RT/100m vs 0.4 BT/100m; ratio = 5.5:1 RT:BT/100m), and Brown Trout (BT) were the predominant species captured below the barrier (mean of 0.8 RT/100m vs 3.4 BT/100m; ratio = 1:4.25 RT:BT/100m) (Table 2). The only observation of a Galaxiid was below the barrier. No other fish species were observed. The total number of fish (all.fish) and the total number of trout (all.trout) tended to be positively associated with the amount of time spent electrofishing within a 100m transect (Fig.A2).

Fish numbers in April 2021: A total of 682 fish were captured and tagged for the first time, and 21 fish were recaptured during the April surveys. The total captures included 387 Two-spined Blackfish, 146 Brown Trout, 170 Rainbow Trout (Table 1). Ten Galaxiids (*Galaxias sp.*) were also captured and released without tagging, including nine above and one below the barrier. More the double the number of blackfish were recorded above compared to below the barrier (mean of 35.8 and 15.7 blackfish/100m respectively). Rainbow Trout were the predominant non-native species captured above the barrier (mean of 12.3 RT/100m Vs. 1.7 BT/100m; ratio = 7.2:1 RT: BT/100m), and Brown Trout were the predominant species captured below the barrier (mean of 9 RT/100m vs. 17.4 BT/100m; ratio = 1:1.9 RT: BT/100m) (Table 2). No other fish species were observed.

Fish numbers in May 2021: A total of 383 were captured for the first time, including 88 Two-spined Blackfish, 81 Brown Trout, 192 Rainbow Trout (Table 1), and four Galaxiids (*Galaxias sp.*; which were only detected above the barrier). During this period, fish were only tagged below the barrier. More blackfish were recorded per transect above compared to below the barrier sites (6.5 and 1.7 blackfish/100m, respectively). While both trout species were recorded on either side of the fish barrier, Rainbow Trout were the predominant species captured above the barrier (mean of 19.2 RT/100m Vs. 5.3 BT/100m; ratio = 3.6:1 RT: BT/100m), and Brown Trout were the predominant species captured below the barrier (mean of 5.3 RT/100m vs. 11.8 BT/100m; ratio = 1:2.2 RT: BT/100m) (Table 2). No other fish species were observed.

Fish Tagging Summary: A total of 939 fish were captured and tagged during the assessment periods (Table 1). This included 415 trout (143 above and 272 below barrier) and 470 blackfish (326 above and 198 below the barrier). The tagged trout numbers include 20 Brown Trout, 123 Rainbow Trout above the barrier, and 184 Brown Trout, 88 Rainbow Trout below the barrier. No Galaxiids were tagged. The majority of the fish were tagged during the April assessment period (a total of 201, 682, and 56 fish were tagged respectively in November, April, and May). Of the tagged fish, 740 were given only PIT tags, nine were given only T-bar Tags, and 190 received both tag types.

Fish Recapture Summary: A total of 161 (17.1%) of tagged fish have been recaptured once within the mark-recapture stream sections during electrofishing surveys (Table 1). This includes 112 (23.9%) of tagged fish recaptured above and 49 (10%) of tagged fish recaptured below the barrier. Furthermore, 86 (60.1%) of tagged trout above and 43 (15.8%) of tagged trout below the barrier were recaptured once in the study period (Table 1 and 3). Similarly, 26 (8.0%) of tagged Blackfish above and 6 (0.3%) of tagged blackfish below were recaptured once. The percentage of recaptured trout and blackfish was less below than above the barrier because only 400m (4 x 100m transects) of the below barrier stream section was surveyed compared to 1200m above the barrier during the May Assessment and Removal Periods. In addition, a lower percentage of blackfish have been recaptured, which might be because the May Assessment and Removal periods primarily targeted trout.

Table 1. *The number of fish tagged and recaptured during each survey period above and below the barrier. The total number of trout and all fish species is given for surveyed transects at both sites. In May, numbers in parentheses indicate fish captured without tagging (they are not included in the Total number of tagged fish but are included in Total*). Trout captured for the first time and recaptured in May were removed from the above barrier site.*

		Tagged Fish					Recaptured Fish		
Site	species	Nov	Apr	May	Total	Total*	Apr	May	Total
Above	Brown Trout	5	15	(59)	**20**	(79)		11	**11**
	Rainbow Trout	26	97	(180)	**123**	(303)	5	70	**75**
	Blackfish	62	264	(88)	**326**	(414)	5	21	**26**
	Total Trout	31	112	(239)	143	(382)	5	81	86
	Total Fish	93	376	(327)	469	(796)	10	102	112
Below	Brown Trout	39	123	22	**184**		8	25	**33**
	Rainbow Trout	9	67	12	**88**		1	9	**10**
	Blackfish	60	116	22	**198**		2	4	**6**
	Total Trout	48	190	34	272		9	34	43
	Total Fish	108	306	56	470		11	38	49
Both	Total Trout	79	302	34	415	(654)	14	115	129
	Total Fish	201	682	56	939	(1266)	21	140	161

Table 2. *The average number of fish captured for the first time and recaptured per 100m transect, during each electrofishing survey period (November 2020, April 2021, May 2021). Fish were tagged and released upon their initial capture, except for trout captured above the barrier in May (shown with an asterisk), which were removed. Total mean is the mean number of fish captured (tagged and untagged) per 100m transect across all sampling periods.*

		Initial Captures				Recaptures				Total Mean
	species	Nov	Apr	May	Mean	Nov	Apr	May	Mean	
Above	Brown Trout	0.4	1.7	4.5*	**2.2**	0	0	0.8	**0.4**	**2.5**
	Rainbow Trout	2.2	11.7	13.8*	**9.2**	0	0.6	5.4	**3**	**11.2**
	Blackfish	5.2	31.0	6.8*	**12.8**	0	0.6	1.7	**1.1**	**13.5**
	No. Transects assessed	12	8.5	13		12	8.5	13		
Below	Brown Trout	3.4	16.4	5.5	**8.4**	0	1.0	6.3	**3.6**	**10.8**
	Rainbow Trout	0.8	8.9	3.0	**4.2**	0	0.1	2.3	**1.2**	**5.0**
	Blackfish	5.3	15.4	5.5	**8.7**	0	0.3	1.0	**0.6**	**9.1**
	No. Transects assessed	11	7.5	4		11	7.5	4		

Fish Weights and Size: Blackfish and Rainbow Trout body lengths and weights were similar above and below the barrier within the mark-recpature survey zones (Fig. 2). However, there was a skew towards smaller Brown Trout above the barrier compared barrier (Fig. 2), even though the largest Brown Trout was also detected above the barrier (maximum length 560mm) (Fig 3; see also Fig. 4 and 5 for other examples of the fish captured).

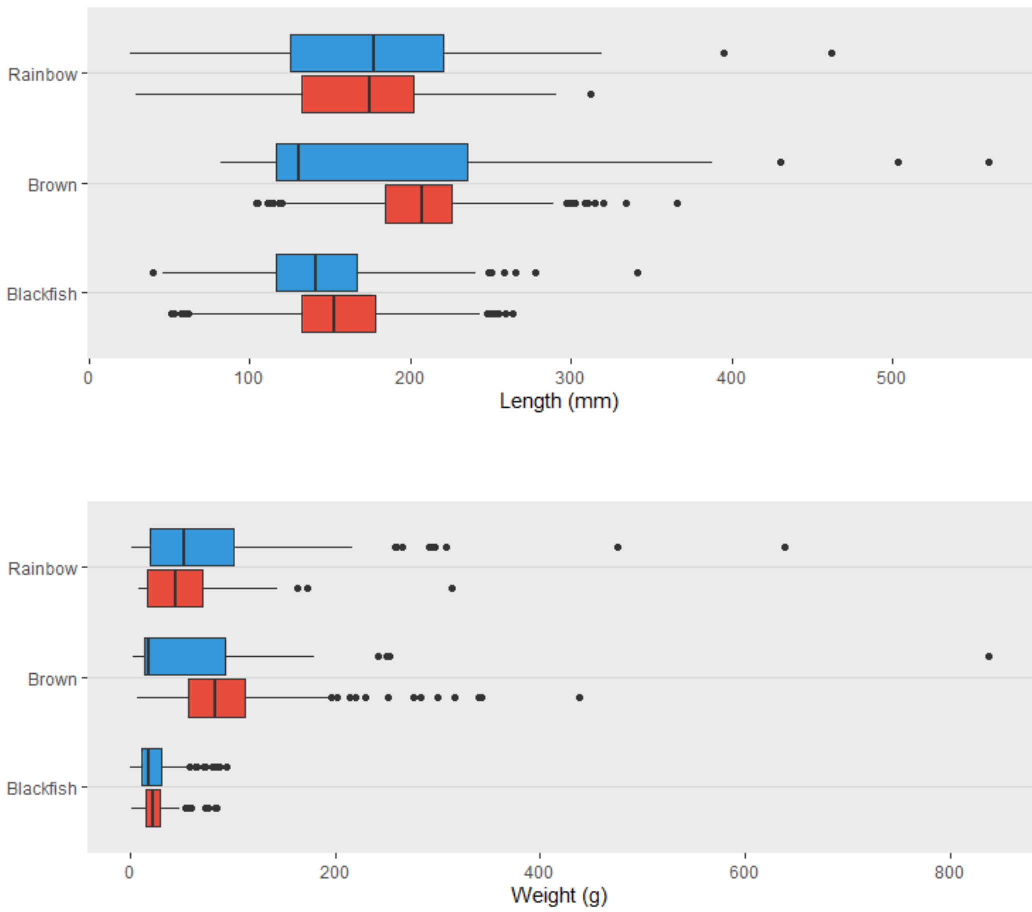

Figure 2. *The length (top panel) and weight (bottom panel) of the three predominant fish species captured above and below the fish barrier within the mark-recapture survey zones. Data is combined for the November 2020 and April 2021 Assessments (May survey results are in Fig. 6). Each coloured box displays the median values and the interquartile range (IQR, which is the range from the first quartile (1Q; 25th percentile) to the third quartile (3Q; 75th percentile)). The lines (or whiskers) indicate the range from the minimum to maximum values (where the minimum is calculated as Q1 – 1.5*IQR and the maximum is calculated as Q3 + 1.5*IQR). The points extending beyond the whiskers are outlier values.*

Figure 3. *A 560mm Brown Trout captured above the fish barrier during electrofishing surveys during the November 2020 surveys.*

Figure 4. *A 240mm Rainbow Trout captured above the fish barrier during electrofishing surveys during the November 2020 surveys.*

Figure 5. *A 142mm Two-spined Blackfish captured above the fish barrier during electrofishing surveys during the November 2020 surveys.*

Summary of Fish Captured in Mark-recapture Transects Before Fish Removals:

Over the three assessments and first removal period, 184 Brown Trout, 88 Rainbow Trout, and 198 Blackfish were captured below, and 79 Brown Trout, 303 Rainbow Trout, and 414 Blackfish were captured above the barrier within the mark-recapture transects (Table 1). This equates to an overall ratio of 0.5:1 Brown to Rainbow Trout below and 3.8:1 Brown to Rainbow Trout above the barrier.

Mark-recapture Analysis:

The mean annual probability of trout survival was estimated to be 0.99 (95% credible interval 0.98 – 1). The capture probability of trout during a single-pass electrofishing survey was estimated to be 0.27 (95% credible interval of 0.22 – 0.33).

Mechanical Fish Removals by Anglers and Electrofishers in Year 1:

A total of 3086 non-native fish were removed from the stream above the barrier during the first year of the management trial. Of these, 71 fish were removed by Anglers, and 3015 fish were removed by Electrofishers (Table 3). Three quarters (74.7%, n=2306) of removed fish were Rainbow Trout, and the others (25.3%, n= 780) were Brown Trout. Anglers caught and removed a similar percentage of Rainbow (80.2%) and Brown Trout (19.8%) compared to those caught and removed by Electrofishers;(74.5% Rainbow, 24.5% Brown Trout). The Anglers conducted removal work along approximately 6km of stream, whereas electrofishing removals were conducted along 13km above the barrier.

Of the removed fish, 11.2% (n=346) were translocated away from the site, and 88.8% (n=2740) were humanely killed. The percentage ratios of Rainbow to Brown Trout were similar for translocated fish (74.6%:25.4%) and humanely killed fish (75.7%:24.3%).

Of the total trout removed, 219 fish (7.1%) were removed from the frog, and fish mark-recapture transects above the barrier. This equates to a mean of 16.8 trout/100m transect (range: 4 - 27 trout/100m) or three Brown and 13.8 Rainbow trout/100m. The other removed trout (n= 2,867) were all from stream sections upstream of the above barrier frog, and fish mark-recapture transects.

Eighty-six (60.1%) of the total trout tagged above barrier (n= 143) were recaptured and removed during the May mechanical removal periods (Table 3). These tagged fish removals included 13 (60.5%) of the total above barrier tagged Brown Trout (n=20), and 73 (59.3%) of the total above barrier tagged Rainbow Trout (n=123). Fifty-four of the tagged trout (eight Brown and 46 Rainbow) were translocated during the electrofishing removal period, representing 37.8% of the total above barrier tagged trout. All three tagged fish captured by Anglers (Table 3) were humanely killed.

When electrofishing removals were conducted along transects on successive days, a mean of 15.3 trout/100m (sd = 3.4 trout/100m) were removed the first day, and a mean of 6 trout/100m (sd = 1.6 trout/100m) the second day. For Rainbow Trout, a mean of 14 individuals/100m (sd = 2.8/100m) and 5.3 individuals/100m (sd = 0.9/100m) removed in the first and second days respectively. For Brown Trout, a mean of 0.7 individuals/100m (sd = 0.9/100m) and 0.6 individuals/100m (sd = 0.9/100m) removed in the first and second days respectively. Two Brown Trout were removed from one of the transects on the second day, even though the species was not detected during the first day.

Removal efficiency varied between the mechanical removal methods. A mean of 5.0 trout were removed per person-hour using electrofishing methods (calculated as 3086 trout removed by 14 people working an average of 44 hours each). A mean of 0.2 trout were removed per person-hour using angling methods (calculated as 71 trout removed by 16 people working an average of 22 hours each). In both, cases the number of fish removed per hour includes project management time, supporting staff time, and fish processing time for all paid and voluntary participants.

Table 3. *The number of non-native fish removed from the stream above the barrier during the first year of the management trial. Tran = fish translocated from the site. H-Kill = fish humanely killed. Tags = number of tagged fish among those translocated or humanely killed. Total = the total number of fish removed and either translocated away or humanely killed (the number includes both tagged and untagged fish).*

	Angler Removals				Electrofisher Removals				Total Removed			
	Tran	H-Kill	Tags	Total	Tran	H-Kill	Tags	Total	Tran	H-Kill	Tags	Total
Brown Trout	5	9	1	**14**	79	687	11	**766**	84	696	13	**780**
Rainbow Trout	14	43	2	**57**	248	2001	72	**2249**	262	2044	73	**2306**
Totals	**19**	**52**	**3**	**71**	**327**	**2688**	**83**	**3015**	**346**	**2740**	**86**	**3086**

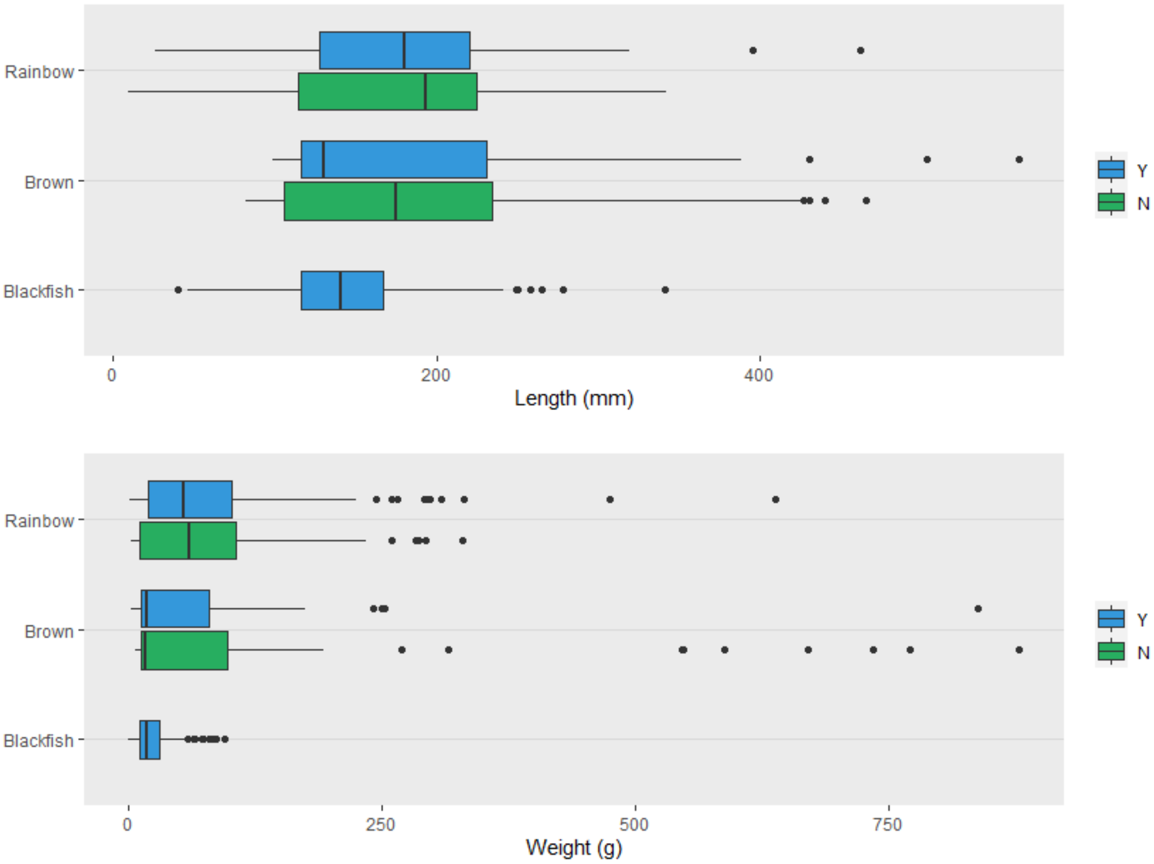

Figure 6: *Comparison of the length (top panel) and weight (bottom panel) of each of the predominant fish species captured above fish barrier within (Y) or outside (N) of the capture-mark-recapture transect area. Box and whisker interpretation can be found in Figure 2.*

Discussion

The survey results provide crucial baseline ("before") data for the 6-year "before-and-after, control-and-impact" (BACI) Management Trial that aims to clarify the impacts of non-native fish on Spotted Tree frog populations. Notably, the November 2020 – May 2021 electrofishing surveys identified which non-native fish species occur at the sites and enabled comparison of the relative abundance of each fish species at sites that can be used to address Management Trial Objectives 1 – 3.

Brown and Rainbow Trout were found to be the main non-native fish species present at the "control" (below barrier) and "impact" (above barrier) management sites. Both trout species are considered to have similar ecological roles, as top-order introduced predators in this stream system, and both species are known to eat Spotted Tree Frog tadpoles (Gillespie & Hero 1999; Gillespie 2001). Surprisingly, invasive European Carp (*Cyprinus carpio*) and non-native Redfin perch (*Perca fluviatilis*) were not detected, despite periodic observations at the sites during the Spotted Tree Frog monitoring program. These other fish are known to eat other native stream-dwelling frog species (Hunter *et al.* 2011) and, when present, are also likely to eat Spotted Tree Frogs.

During the November 2020 – May 2021 surveys, 415 trout were tagged (marked) before removals commenced. This single-pass electrofishing survey data can already help approximate the potential "before" removal trout abundance at sites. Single-pass count data are generally highly correlated with abundance estimates derived through multi-pass sampling methods that assess capture probabilities to account for imperfect fish detection (e.g. Kruse, Hubert & Rahel 1998; Hanks, Kanno & Rash 2018). Trout electrofishing capture probabilities can be influenced by multiple factors like operator experience, stream flow, width, depth, and habitat complexity, and can range between at least 0.17 (Korman *et al.* 2009) to 0.84 (Hanks, Kanno & Rash 2018). The mean trout capture probability using single-pass electrofishing methods during the November 2020 – May 2021 survey period was 0.27 with a 95% credible interval of 0.22 – 0.33. Trout abundance can be estimated by dividing the single-pass survey counts by the capture probabilities (e.g. Kéry & Schmidt 2008). Therefore, based on the maximum of the mean April and May counts, and using the 95% confidence interval capture probabilities, there could be around 7 – 11 Brown Trout and 34 – 51 Rainbow Trout/100m above the barrier, and 33 – 49 Brown Trout and 15 – 23 Rainbow Trout/100m below the barrier. These estimates will be further refined following the next round of fish surveys and second-year trout removals using mark-recapture and depletion sampling methods.

Rainbow Trout were the most abundant non-native fish species above the barrier, and Brown Trout were the most abundant non-native fish below the barrier during the "before" removal period. This result was somewhat surprising, as we had assumed that the trout populations would be similar at each site. Trout abundance and densities can be influenced by multiple factors including stream size, flow, and structure that affect the site-specific habitat suitability for each trout species (e.g. McRae & Diana 2005), predatory and competitive interactions between co-occurring species (e.g. Blanchet *et al.* 2007) and potentially differences in recreational fishing pressure at sites. The "impact" (above barrier) site tends to be narrower and has more shaded stream sections compared to the "control" (below barrier) site. These conditions also mean that the control site is easier to access and therefore subjected to more intensive fisher pressure from anglers. Notably, to date, nine T-bar-tagged fish have been reported by members of the public and only from below the barrier (all fish were released at the capture site, although five tags were removed). Because there are differences in the relative abundance of each trout species above and below the barrier, further investigation may be required to understand the relative effect of each trout species on Spotted Tree Frog populations.

The size and age of trout may influence predation risk for tadpoles. For instance, juvenile Brown Trout appear to eat Spotted Tree Frog tadpoles at a faster rate than adult Brown Trout (Gillespie 2001). Juvenile trout may also be more dangerous for tadpoles, as smaller trout can more easily access the shallow microhabitats used by tadpoles when compared to larger trout (Gillespie 2001). The November 2020 – May 2021 survey results revealed that Rainbow Trout weights and sizes were similar between the sites. However, Brown Trout tended to be smaller above the barrier compared to below the barrier. But despite this, Spotted Tree Frog recruitment rates in recent years have been higher above than below the barrier (West et al in prep). Thus, knowledge of the abundance of the age and size classes of the trout species within the control and impact sites may be necessary for clarifying trout predation effects on Spotted Tree Frog populations at each location.

In the first year of the Management Trial, anglers and conservation practitioners collaboratively removed 3086 trout within the first 13km of the stream above the fish barrier. The removed fish included 2306 Rainbow Trout and 780 Brown Trout. Given that trout could occupy around 29km stream above the barrier and electrofishing removals are imperfect, there were likely to be more than double this number of trout before the fish removals.

By extrapolating the above barrier pre-removal trout abundance estimates per 100m to the 29km stream section, there could have been around 12,000 – 18000 fish (or 2,200 – 3,300 Brown Trout and 9800 – 14,700 Rainbow Trout) "before" the removals. These total abundance estimates should be interpreted with caution, particularly as the trout population densities may vary spatially and be influenced by factors including stream temperature, width and depth, and micro-habitats types available within stream sections. Notably, the stream width continues to narrow above the area from which trout were removed in the first year, and consequently, these abundance estimates may be excessive. The abundance estimates will be refined in the program's second year using mark-recapture and depletion sampling methods.

The first aim of the Management Trial is to evaluate the effectiveness of the mechanical removal methods. Assuming the total "before" removal trout population abundance above the barrier is between 12,000 – 18000 fish, 17 – 25% of fish may have been removed in the first year. In other studies, ten consecutive electrofishing removals campaigns were required over 3 years to remove 100% of Brown Trout from streams that were up to 5km in length (Carosi, Bonomo & Lorenzoni 2020). The electrofishing methods, which removed the majority of trout (n=3015) at a rate of 5 fish/person hour, have been more effective than the angling removal methods, which removed 71 trout at a rate of 0.2 fish/ person hour. The Angler fish removal rate is not surprising given the relatively narrow stream width and extent of overhanging and fallen vegetation that made site access and fishing difficult. Angler involvement in the removals has been essential for community engagement, enabling recreational fishers to assess the site's value for recreational fishing and learn about the challenges involved in protecting the frogs. Angling methods may be used in subsequent years. However, following consultation with Recreational Fishing Groups, the Management Committee has recommended that conservation practitioners and anglers focus their collaborative efforts on the electrofishing removal work.

The success of this Trial partly depends on the effectiveness of the concrete fish barrier. Inspection of the fish barrier in November 2020 indicated that some repairs were required to ensure that upstream fish movement could be prevented during high streamflow events. Notably, the inspection revealed that a deep pool had formed in front (downstream) of the barrier, allowing fish to jump over the barrier. In addition, rock on the left-hand side of the barrier could act as a ladder for fish movement in high flow events. Funding was obtained to repair the barrier, and repairs were completed in September 2021. Several high flow events have occurred following the May 2021 removals, and some upstream fish movement may have occurred. Fortunately, fish movement across the barrier in either direction can be assessed through mark-recapture surveys and analysis.

While the primary focus of the Management Trial is to help the Spotted Tree Frog, other native freshwater taxa may also benefit from non-native fish removals. Trout are recognized as an important threat to Australian native fish and some freshwater invertebrates (Jackson 1981; Crowl, Townsend & McIntosh 1992; Lintermans *et al.* 2020). In particular, trout can reduce small native fish populations by eating and out-competing species, like the Barred Galaxias (Raadik, Fairbrother & Smith 2010). Galaxias were rarely detected during the fish assessments and removal periods. In contrast, Two-spined Blackfish were frequently detected, and almost as many Blackfish (total n = 525) were encountered as the two trout species combined (total n= 630, includes 415 tagged + 215 removed). By monitoring native fish in this system during the planned non-native fish removal trial, we can investigate relationships between non-native and native fish.

Electrofishing and angling methods have been used to successfully control and eradicate trout from rivers elsewhere (Rytwinski *et al.* 2019; Carosi, Bonomo & Lorenzoni 2020), including in other streams within Australia (T Raadik ARI *pers comm*). However, intensive and successive mechanical removal treatments are required, particularly if trout must be eradicated to ensure native species population recovery (Rytwinski *et al.* 2019; Carosi, Bonomo & Lorenzoni 2020). Recently mechanical (electrofishing) removals over 10 years successfully eradicated two trout species (Brook Trout, *Salvelinus fontinalis*, and Brown Trout) from mountain streams in central Spain, and enabled the recovery of another stream-dwelling amphibian (Iberian Frog *Rana iberica*) (Bosch *et al.* 2019). While trout eradication may ultimately be required to elicit a measurable increase in the Spotted Tree Frog population, we currently aim to reduce trout densities rather than eliminate trout. If successful, we anticipate the reduced trout predation pressure will allow an increased number of tadpoles to survive through to the 1-year old life-stage compared to pre-removal frog numbers. Therefore, surveys in the 2nd – 6th years will focus on evaluating changes in 1-year-old frog abundance. Adult frog maturation takes around 3-4 years at these sites (Gillespie 2010; Gillespie 2011), which means changes in the adult Spotted Tree Frog population will only occur from the 4th – 5th year after trout removals. Given that trout population numbers are expected to rebound through breeding activity, trout removals are required in each year of the Management Trial.

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Appendix 1

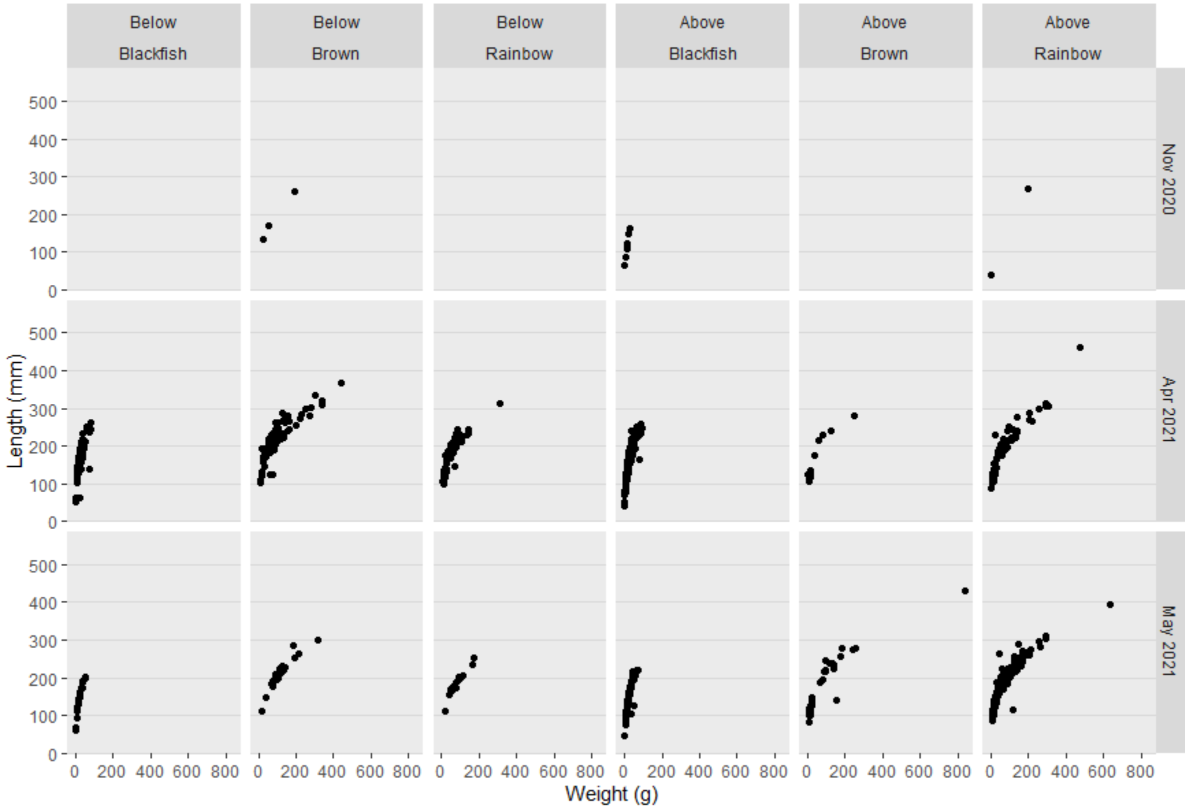

Figure A1. *Length versus weight for Blackfish, Brown Trout, Rainbow Trout captured (and measured and weighed) during the November 2020, April 2021, and May 2021 survey periods. Not all captured fish were both weighed and measured during surveys.*

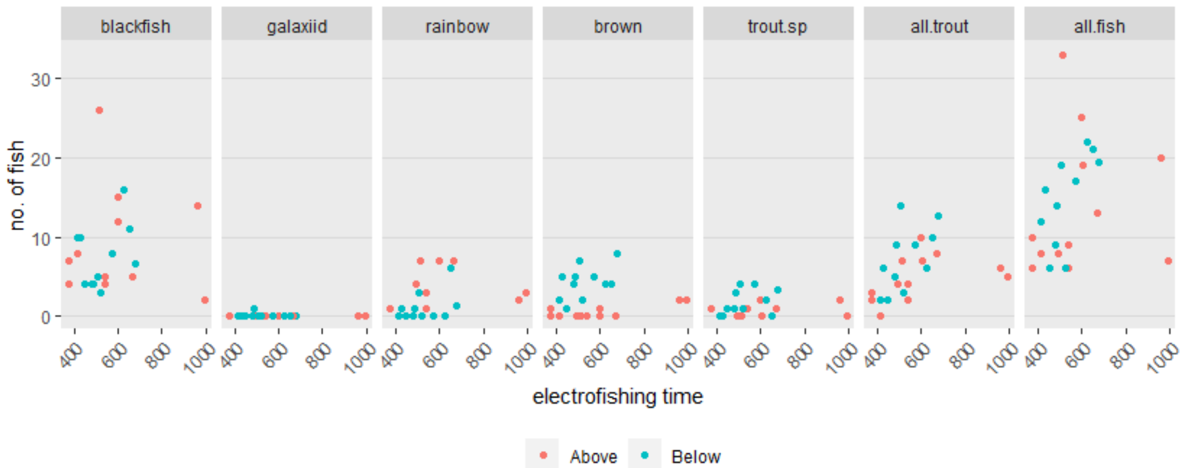

Figure A2. *Comparison of the number of fish captured relative to the time spent electrofishing each of the 100m transects above and below the fish barrier. Each point represents the number of fish captured for a 100m transect, with red points indicating transects above the barrier and blue points indicating transects below the barrier. Trout.sp is the number of trout observed that were not identified to a species level. All.trout and All.fish are the total number of trout and fish observed, respectively.*

Further information:

http://www.nespthreatenedspecies.edu.au

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