Update on saving Running River Rainbowfish

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Project background

Various rainbowfish populations across the Burdekin River system have long been recognised as looking different, with the most distinct populations referred to as Burdekin Rainbowfish as reviewed by Martin & Barclay (2016). On the basis of our research we now specifically separate the population from Running River as being distinct from Burdekin Rainbowfish and thus call it the Running River Rainbowfish (Figure 1). Running River Rainbowfish have long been informally known as a unique population since Ray Leggett first collected them in 1982 as part of his survey work in the Burdekin River system (Leggett 2004; Martin & Barclay 2016). They quickly became well known due to their bright colours and patterning and have been maintained within the aquarium hobby ever since. The current part of the story began in August 2015 when Michael Hammer and I visited Running River. We observed that Eastern Rainbowfish (*Melanotaenia s. splendida*) had been introduced into an area upstream of where Running River Rainbowfish currently occurred (Figures 2–4) – an area that was previously devoid of any rainbowfish – and that they were starting to move downstream (Unmack & Hammer 2015). We quickly surmised that this invasion posed a high risk to Running River Rainbowfish and could potentially result in the extinction of this population genetically through introgression. Thus, a number of live fish were collected and transported back to the University of Canberra as a security measure. At that point we

Figure 1. Male Running River Rainbowfish. Photo: Steven Hume

enlisted ANGFA's assistance with running a crowd funding effort to try and establish a captive population based on fish with known genetic origins (i.e., excluding any hybrids). Genetic research is expensive and alternative funding sources are not easily available at short notice, thus the crowd funding effort was the key to being able to make this project possible. Initial genetic results showed that Running River Rainbowfish is quite distinct to other rainbowfish populations across the Burdekin River system and the project quickly switched to saving the species in the wild. Options to ultimately reverse the introduction of Eastern Rainbowfish into Running River are complex from both a logistics and ethical standpoint, requiring coordination and permissions at all levels of government, time and great expense. However, due to the current level of limited funding and urgent need to act now, short term

Figure 2. Map showing location of various locations mentioned in the text.
Map data provided by the following sources: Google, CNES/Astrium, DigitalGlobe and CNES/Spot Image.

solutions have presented themselves which could enable the establishment of new secure populations in creeks above known fish barriers, within the Running River catchment. Fortunately we have identified two suitable creeks and we have been breeding Running River Rainbowfish for translocation. The remainder of the article outlines in more detail the efforts to conserve Running River Rainbowfish.

Collaborators and contributors

We have been fortunate to draw on a wide variety of support to help make this project possible. Firstly none of this would have been possible without the incredible generosity from rainbowfish people from around the world; the support from everyone for the crowd funding portion of the project has been amazing. In the race to save this fish from extinction, Diversity Arrays Technology – based at the University of Canberra – have provided all of the genetic data on their fast track to provide information as quickly as possible. The project has benefited greatly from our research team examining broader rainbowfish systematic research: Keith Martin (who was the initial cause of all this with his incessant poking around in nooks and crannies for interesting rainbowfishes), Michael Hammer, Mark Adams, Culum Brown and Gerry Allen. Many others continue to provide valuable contributions. From the University of Canberra: Michael Jones, Mark Lintermans, Arthur Georges and Bernd Gruber. James Cook University: Jason Schaffer and Damien Burrows. Flinders University: Luciano Beheregaray, Catherine Attard and "Yuma" Sandoval-Castillo. Queensland Fisheries: Steven Brooks and the Australian Wildlife Conservancy: Eridani Mulder and John Kanowski.

Crowd funding

ANGFA's crowd funding effort was a massive success with AUD$10,605 raised to date! This has been a fantastic response from the worldwide rainbowfish community that has allowed us to greatly expand on what the project has already accomplished. Table 1 provides a breakdown of the countries and sources of donations,

Table 1. Sources of funding (in AUD$) to date for the Running River Rainbowfish crowd funding effort.

Country	Total	Source	Total
USA	5341	club	6635
Australia	2985	individual	2820
Switzerland	1100	company	1150
Canada	794		
Germany	385		
Total	**10,605**		**10,605**

Table 2. Donors to the Running River Rainbowfish crowd funding effort. Donor names in bold gave $500 or more, the two underlined names gave $1000 or more!

Clubs	Individuals	Businesses
Australia	Matt Alderton	**Aquariums By Design, Greg Martin (Aust)**
ANGFA New South Wales	Stefan Anderson	Reef to Rainforest Media, James Lawrence (USA)
ANGFA Victoria	Gregory Andrews	**Valleyfish Inc., Thomas Townsend (USA)**
Canberra Districts Aquarium Soc.	Earl Blewett	
Eastern Districts Aquarium Soc.	Christine Borthistle	
	Andrew Clarke	
Europe	Rick Datodi	
International Rainbowfish Group	Claudia Dickinson	
	Peter Eggler	
North America	Graeme Finsen	
Aquarium Club of Edmonton	Doug Harrison	
Canadian Assoc. of Aquarium Clubs	Mike Helford	
Champaign Area Fish Exchange	Dave Howarth	
Columbus Area Fish Enthusiasts	Michael Kurhne	
Durham Region Aquarium Soc.	Gary Lange	
Greater Cincinnati Aquarium Soc.	Andrew Martin	
Greater Seattle Aquarium Soc.	Tan Ng	
Green Bay Aquarium Soc.	Charles Nunziata	
Milwaukee Aquarium Soc.	David Roberts	
Missouri Aquarium Soc.	David Roy	
Oklahoma Aquarium Soc.	Konrad Schmidt	
Pittsburgh Aquarium Soc.	Rachel Shen	
Raleigh Aquarium Soc.	Graham Thompson	
Sacramento Aquarium Soc.	Sumer Tiwari	
	Peter Warth	
	Dave Wilson	

Table 2 includes a list of donors by individuals, clubs and businesses. As of September 10, 2016, there were 51 donations; 27 from individuals, 21 from clubs and three from companies. Almost half of the money came from North American fish clubs ($4805), while $4440 came from six donors highlighted in Table 2! So far this has enabled us to sequence 207 fish (at a cost of $7267); this includes 79 wild captive fish from pre-existing populations, 84 live wild fish and 44 frozen wild fish from various parts of Running River. Sequencing the fish has been critical for determining the purity of our wild collected individuals that are used to breed fish for reintroduction. Funds are now being used to cover the costs of shipping fish from Canberra to Townsville to ongrow them for reintroduction into the wild. We plan to continue our crowd funding effort and expand it to include two additional rainbowfishes, Malanda Rainbowfish and Lake Eacham Rainbowfish as both are in serious trouble in the wild and need genetic research to define the status of their populations. More details on these projects will be provided in future issues of Fishes of Sahul.

What is Running River Rainbowfish?

When we discovered that Running River Rainbowfish were in trouble (Unmack & Hammer 2015), we had no concrete information on their taxonomic status. The recent review on rainbowfishes in the Burdekin system (Martin & Barclay 2016) really helped to clarify the occurrence and distribution of different forms in this system. Essentially there are populations of rainbowfish in the Burdekin that are Eastern Rainbowfish, but there are also a group of populations called Burdekin Rainbowfish that have a collection of traits that make them stand out as being different to Eastern Rainbowfish; but how different has remained an unanswered question, until now. Preliminary examination of high resolution nuclear genetic data suggests Running River Rainbowfish is distinct relative to other rainbowfishes in the Burdekin system. Initial examination of morphology by Gerry Allen and Michael Hammer also found some traits that differ. Further work is continuing which should lead to the species being described and named in the near future.

Captive genetic results

We examined captive fish from six people who had existing stock of Running River Rainbowfish. We examined 8–10 fish from most stocks and 35 fish from one source. Some fish were from old collections: 1997 (one remaining live fish) and 1998. Some were more recent collections: one from 2010, two from 2012, and one where the date and origin of the fish was unknown. Two of those populations (the one fish from 1997 and the unknown date) were not pure Running River Rainbowfish. All un-pure fish were removed from any breeding. One breeder's fish had strong deviations from wild fish probably as a result of inbreeding (new individuals have since been incorporated into their breeding population to bring genetic diversity back up); other populations were consistent with wild fish.

Hybridisation in the wild

It is clear that the number of hybrids in the wild, while still low, is increasing. There are also biases in what fish we have tested; most have been larger adult fish for our breeding program which are less likely to be hybrids due to the short time that Eastern Rainbowfish

Figure 3. Running River junction with Puzzle Creek. Photo: Steven Hume

Figure 4. Introduced Eastern Rainbowfish collected from Running River at junction with Puzzle Creek, female above, male below. Photo: Steven Hume

have been present (first detected in August 2015 by Unmack & Hammer). Upper portions of the river closer to where the introduced Eastern Rainbowfish are present should have more Running River Rainbowfish hybrids – as they are closest to the invasion front – than populations at the far end of the reach. Identifying hybrid fish in the wild is difficult, especially females (Figure 5), although we expect that with sufficient closer examination of larger numbers of fish that hybrids will become easier to identify. We genetically tested 11 fish that I collected and preserved in 1997 as our "control" sample to compare to new collections. We tested 52 live fish plus six preserved samples collected in August 2015 (we did however observe a number of Eastern Rainbowfish living along side them which we specifically avoided), all were pure Running River Rainbowfish based on genetic comparisons to rainbowfishes from across and beyond the Burdekin River system. Of the seven live male fish collected in November 2015, one was an F1 hybrid. We examined additional fish collected in February 2016, from the upstream and downstream extent of their known distribution within the the Running River and noticed many fish from the upper site looked odd. We then sequenced five "odd" fish and five "pure" fish, plus 10 small (~1.5 cm) fish. Of those, two "odd" fish proved to be F1 hybrids and two were backcrosses between F1 hybrids and Running River Rainbowfish. One of the "small" fish was an F1 hybrid. Thirty-two larger live fish from the lower site on Running River (Figure 5) were genotyped for captive breeding, two of those were female F1 hybrids (Figure 6), however it is clear that of the additional ~170 untested live fish that some are almost certainly hybrids. Additional preserved samples were collected in August 2016 but have not been genetically tested. Again, upper sections of the river seemed to have more "odd" looking fish, while those from the lower section mostly looked good (keeping in mind that our ability to pick hybrid fish in the wild may not be highly accurate especially as backcrossing progresses). It is important to note that we have only sequenced a small number of the fish we have preserved, thus if future funding becomes available we will be able to clarify hybridisation patterns with less bias (note that we have only used the crowd funding money to address immediate

Figure 5. Running River just above the lower gorge. Photo: Steven Hume

questions relating to Running River Rainbowfish conservation – not broader research questions).

Fish breeding

As a result of the genetic sequencing, we now have evidence consistent with Running River Rainbowfish being a distinct species; a species under threat from hybridisation, and found only in a single habitat with a very restricted distribution. We initiated a breeding program (Figure 7) using the wild genotyped fish to breed fish for release into nearby tributaries of Running River; namely Deception and Puzzle creeks. By doing so we aim to establish wild populations that are safe from invasion by other rainbowfish (and hence hybridisation), and provide the species with a new home where it's genetics will remain pure. We plan to start releasing fish in October/November of 2016. All translocation efforts are being closely coordinated with Queensland Fisheries.

Once we knew that our initial 26 pairs of wild fish collected in August 2015 were pure we paired them all up with the help of Luciano Beheregaray's lab at Flinders University. Luciano's lab group ran a series of analyses

Figure 6. The two wild female F1 hybrids identified by DNA sequencing from Running River just above the lower gorge (Figure 5).
Photo: Michael Jones

to allow us to pair up individual fish to maximise the genetic diversity between them and to reduce any chances of full or half sibling matings to minimise inbreeding. The other key point about breeding fish for conservation is to get an even number of offspring from each pair to ensure the genetic diversity from each individual fish is well represented in the translocated populations. This meant collecting mops from 26 pairs all in individual 50 litre aquariums and then transferring spawning mops for a week worth of breeding to 26, 20 litre aquariums, and raising those fish until they were large enough to be moved to larger aquariums. Each week of spawning required an additional 26 aquariums to house the new fry in! We raised approximately 110 fish from each pair of fish for a total of about 2800 fish. We have subsequently added 24 new breeding fish from our February 2016 collection and switched to breeding fish in groups of four fish (two pairs) per aquarium due to space limitations and because the fish settle in better when four are present vs. two. We are hoping to raise another 2000 or so fish for release.

Fortunately we have had terrific help from Jason Schaffer and Damien Burrows in TropWATER at James Cook University, Townsville. They have been providing all of the on-growing facilities and personnel to take care of fish. Once fry at the University of Canberra are large enough we have been shipping them up to Townsville as they have much warmer conditions to on-grow the fish prior to their release (Figure 8).

Finding new homes for Running River Rainbowfish

Peter Unmack, Steve Hume, Jason Schaffer and Mark Lintermans visited the Running River region on Febuary 27–28, 2016 (Figure 2). The primary goal was to try and determine whether some tributary creeks might hold Running River Rainbowfish and/or be suitable as translocation sites for Running River Rainbowfish to ensure they can persist in the wild. With the terrific help of Eridani Mulder from the Australian Wildlife Conservancy we sampled parts of Puzzle and Deception creeks as well as collecting more rainbowfish from Running River. We determined that both creeks would be suitable translocation sites based upon the presence of substantial waterfall barriers, areas of permanent water and the lack of existing rainbowfish populations.

Figure 7. Running River Rainbowfish breeding setup at the University of Canberra. Photo: Michael Jones

Deception Creek is a major tributary that enters Running River below the lower gorge (Figure 2). The lower section of Deception Creek flows through a rugged gorge, before entering the lower plains and meeting Running River. We sampled three sites in the mid reaches, from the uppermost permanent water (Figure 9), a site at the upper end of the gorge section with a waterfall (Figure 10) and one site in between.

Figure 8. Jason Schaffer putting fish into ponds at James Cook University. Photo: Glenn Morga

Figure 9. Deception Creek, upper most permanent water. Photo: Steven Hume

Only one fish species, Spangled Perch (*Leiopotherapon unicolor*) was found at all sites examined. Several Saw Shell Turtle (*Myuchelys latisternum*) were observed along with a *Cherax* crayfish and *Paratya* shrimp, all of which suggests that water is fairly permanent in this system.

Puzzle Creek is a major tributary that enters Running River in the middle of the upper gorge (Figure 2). Most of the lower half of Puzzle Creek flows through rugged gorges with several major waterfalls of up to ~20 m, before dropping over one last waterfall and meeting Running River a few hundred metres downstream (Figure 3). We sampled the lower reaches of Puzzle Creek (Figure 11) to just above the lowermost waterfall and the uppermost permanent water. Only Spangled Perch were found in the lower reaches. Despite introduced Eastern Rainbowfish being present at the mouth of the creek, none were found further up Puzzle Creek as it has a steep gradient just upstream of where it drops into the upper Running River gorge. No other fish were observed, however, Purple Spotted Gudgeon (*Mogurnda adspersa*) were probably present in the lower reaches as well. We also sampled the uppermost permanent waterhole on Puzzle Creek at night and captured abundant Purple Spotted Gudgeon and *Macrobrachium australiense* shrimp. Spangled Perch were not captured, but are known to be present.

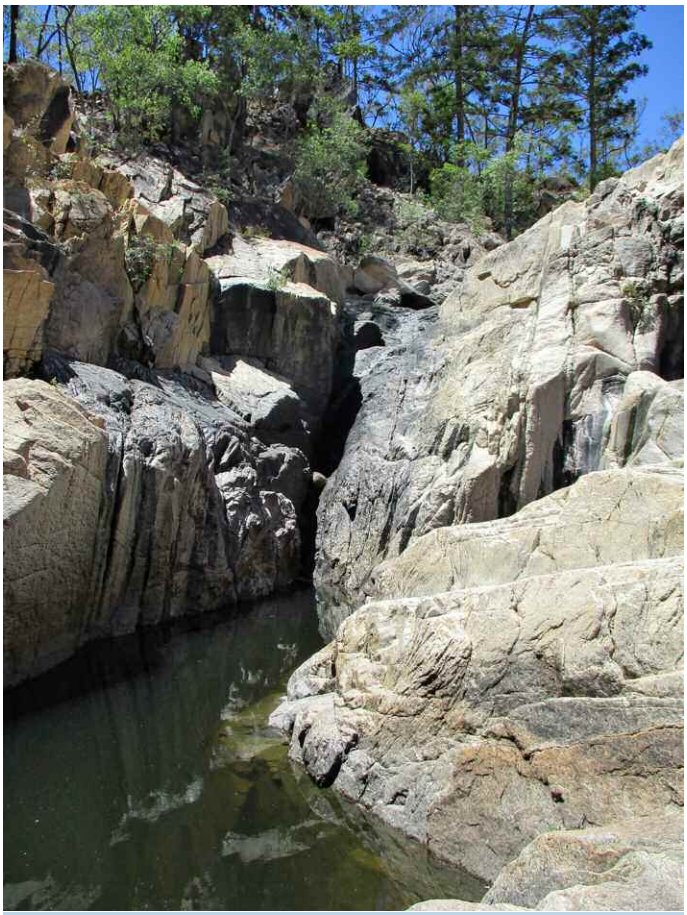

Figure 10. Waterfall in Deception Creek, approximately 10 m high. Photo: Mark Lintermans

Future actions

Karl Moy from ANGFA Queensland has just moved to

Figure 11. Lower Puzzle Creek below the lowermost waterfall. Photo: Mark Lintermans

Canberra to start his masters degree at the University of Canberra studying Running River Rainbowfish. The two primary goals are to better understand the hybridisation process between Running River Rainbowfish and Eastern Rainbowfish through mate choice experiments. The second goal is to determine how we can improve survival success of aquarium raised rainbowfish being reintroduced into Puzzle and Deception creeks. We are hoping to start releasing fish in late spring 2016. Hopefully by March 2017 or so we'll have some indication of whether Running River Rainbowfish appear to be becoming established in these new creeks. In the longer term it would be good to be able to establish additional populations if suitable creeks can be identified in order to ensure their long term conservation.

Running River Rainbowfish eggs from genotyped wild fish have been distributed to ANGFA people in various states. In addition there are pre-existing captive populations that have been confirmed pure by DNA sequencing that are now being bred, thus they should become more widely available in the aquarium hobby. The fish is now starting to become more widely available in Europe through the IRG and they will soon be shared with hobbyists in North America. Anyone wishing to visit the section of Running River with Running River Rainbowfish on Zig Zag station should be aware that there is no public access to the river. In addition, Zig Zag station has just been sold to a new owner. It is important that anyone wishing to visit the river contacts the land holder in advance to avoid any issues. I'd be happy to put people in contact as needed. Both Puzzle and Deception creeks are also on private land owned by the Australian Wildlife Conservancy with restricted access. This limited access will help protect these translocated populations.

The future of Running River Rainbowfish is far from secure, but these first steps are crucial to success. The next 6–12 months will determine whether our efforts are successful in the short term. We will continue to report on the project in Fishes of Sahul as new information becomes available.

References

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A male Empire Gudgeon and the eggs he fertilised on a leaf. Behind him you can see the female fish amongst the plants. Photo: Dave Wilson

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