Delivering a targeted e-water regime to support a higher level of Southern Bell Frog *(Litoria raniformis)* recruitment in temporary wetlands, Riverland SA

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1 Overview

The natural water cycles of wetland ecosystems in the Murray-Darling Basin are historically dynamic, with regular variations in water levels and varied water flows over time (Kingsford 2000; Pressey 1990). Variable water levels create ideal conditions for natural wetland biological processes to occur, and this helps to make available nutrients and food that support several species, including frogs, fish, and birds (Walker & Thoms 1993).

Concomitantly, regular high flow events are critical in supporting vegetation settlement and growth (Rogers & Ralph 2010) and initiating reproductive cues used by some frog species (Spencer & Wassens 2009). However, the creation of locks and weirs along the Murray-Darling River systems has caused a lack of natural water flow and habitat fragmentation, dramatically impacting the health of wetland ecosystems (Bunn and Arthington 2002; Kingsford 2000; Walker & Thoms 1993). With the alteration of natural water cycles, the reduced frequency and duration of natural floods have led to many wetland ecosystems to be inundated permanently, or disconnected entirely from the main river (Pressey 1990). Wetlands that are inundated permanently are known to be less ecologically productive due to the lack of varying water levels which, in the past, would have coincided with the natural river flows.

On the contrary, wetlands that disconnect permanently from the main river (temporary wetlands) do not receive water unless there is a high flow (Brock *et al.* 2003; Boulton & Brock 1999). The reduced frequency of natural floods means that temporary wetlands do not receive water for an extended period. This is having a negative impact on the many fauna and flora species that rely on natural inundation to survive, including long-lived vegetation health and breeding cues for frog species (Brock *et al*. 2003; Boulton & Brock 1999). The change in the water cycle has negatively impacted the ecological health of wetlands all along the Murray River. Unless alternative strategies are used to deliver water to these threatened habitats, they will permanently lose the services and wildlife they have historically supported.

The Murraylands and Riverland Landscape Board (MRLB) partnered with the Commonwealth Environmental Water Holder (CEWH) to mitigate the effects of altered flows by actively delivering water to the environment, specifically to temporary wetlands across South Australia's Riverland region. Environmental water (e-water) is water managed to maintain or improve the ecological health of rivers and wetland systems, including the native flora and fauna that rely on them (Pittock & Lankford 2010). By maintaining the health of these systems, they will continue to support communities and future generations. Specifically, over the past 15 years, e-water plans have been developed annually to facilitate water delivery to temporary wetlands in the Riverland. These temporary wetlands (wetlands situated above normal river level) historically received water from high flow events to inundate the entire wetland (Kingsford 2000; Boulton & Brock 1999). The supply of e-water has allowed these wetland ecosystems to persist where they would have dried with no intervention. When timed accurately, the delivery of e-water can promote many ecological benefits to temporary wetlands, including the growth of aquatic vegetation.

The delivery of e-water to temporary wetlands during the springtime can encourage the growth of a wide range of emergent and aquatic vegetation that many species rely on as a food resource or habitat (Hoffman 2018). In particular, e-water regimes have been beneficial for frog species as newly inundated areas promote successful breeding and recruitment (Ocock *et al.* 2013, Mann *et al.* 2010). In 2014/2015, a study conducted in the Riverland found that frog breeding (i.e., the presence of tadpoles in early development stages) was 99% more successful in temporary wetlands compared to wetlands that were connected permanently to the river, i.e. connected at pool (Hoffman 2018). In addition to the support of essential habitats and food resources, due to the temporary nature of these wetlands, it is thought that they may minimise aquatic predation pressures (e.g. fish and yabbies) on embryonic and larval frogs (Hoffman 2018). Further, a comparison of sampling data to the high flow events in 2016/17 in the SA Murray River wetlands, frog recruitment (i.e. the progression of tadpoles in early development stages into juvenile frogs) was much more successful in years of e-water delivery due to the absence of fish and crustaceans in temporary wetlands (Hoffman 2017).

Of the 12 frog species known to occur in the Murray Valley of South Australia, the Southern bell frog (*Litoria raniformis*) is considered to be the largest (Tyler & Walker 2012) and occupies both permanent and temporary wetlands, predominantly the latter for breeding and larval development (Wassens et al. 2008; Schultz 2007). It is also known as the 'growling grass frog' because the male's unique mating call which consists of a long, loud growl, sometimes followed by a few short grunts (Pyke 2002). Breeding generally occurs in the warmer months (September to April) and is triggered by flooding or a rise in water levels. Once a male has successfully bred with a female, it takes only a few days for egg-laying to occur and 2-4 days later, tadpoles will hatch (Pyke 2002). Compared to other frog species, *L. raniformis* has one of the most prolonged and complex metamorphosis processes extending over three months and sometimes up to 12 months over winter (Pyke 2002). During this time, adequate food and habitat resources are required for optimal tadpole development (Wassens *et al.* 2008). This species' distribution and abundance have greatly diminished over recent years leading to its current conservation status as a vulnerable species under the *Environment Protection and Biodiversity Conservation Act* (1999). One of the key factors contributing to this species conservation status is the lack of regular flooding.

While the delivery of e-water has been demonstrated to improve recruitment significantly in amphibians in the South Australian Murray-Darling Basin (SAMDB), the recruitment of *L. raniformis* was significantly low (Hoffman 2018). The need to investigate the requirements of *L. raniformis* to improve breeding success led to the consideration of alternative water regimes. In 2019/2020, a pilot study was conducted, which involved the delivery of e-water to temporary sites in the Riverland and deviating from the conventional water regime at some locations. The conventional regime commonly deployed for temporary wetlands in the SAMDB includes filling the wetland to a nominal 'full' level, delivered via pumping, and refilling once levels have drawn down to approximately half full to one-third through evaporation. Multi-method surveys conducted in 2019/2020 revealed that e-water would need to be maintained to a higher level to inundate frog and tadpole habitat (fringing and emergent vegetation) and food resources during the three month *L. raniformis* tadpole metamorphosis process. During this pilot study, 2 of the 5 wetlands that received additional top-ups due to the presence of *L. raniformis* tadpoles, resulting in a 100% increase in tadpole recruitment compared to the other wetlands.

Overland Corner was one of the wetlands that received additional top-ups and had a higher recruitment success due to the low percentage of other aquatic fauna caught, such as fish and crustaceans (Figure 1). These species are known to enter through pumps and reproduce over time, having significant predation and competing impacts on tadpole abundance (Hoffman 2015 and 2017). Although the calling behaviour of *L. raniformis* was detected at wetlands such as Molo Flat, a high level of predation and competition was evident at the site shown by the extremely low numbers of tadpoles caught (Figure 1), which meant additional top-ups were not warranted. This particular wetland had a higher number of other aquatic fauna caught as it had already been pumped prior to early spring. While the 2019/2020 pilot study provided evidence that maintaining high water levels in wetlands that had an absence of competing species promoted *L. raniformis* breeding/recruitment, further research was required to confirm the success of targeted water regimes in temporary wetlands.

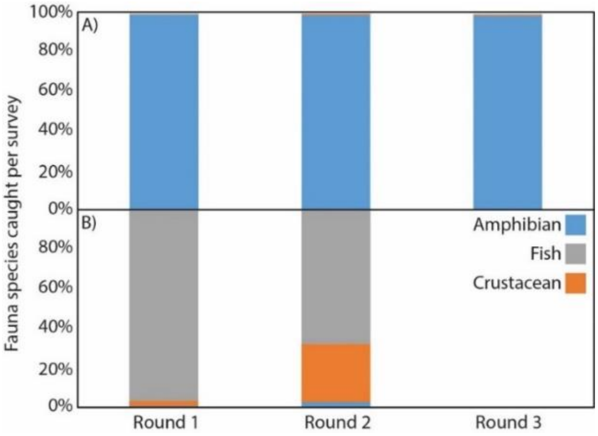


Figure 1. The proportion of fauna species caught during each survey round at Overland Corner (top) compared to Molo Flat (bottom) in 2019/2020

In 2020/2021, conventional e-water regimes were delivered alongside novel, targeted water regimes to seven temporary wetlands using pumping infrastructure, expanding on the 2019/2020 trial results. The wetlands selected for this study were located in clusters to encourage movement between them, as recent studies have shown that this can ultimately lead to lower site fidelity, with individuals moving smaller distances (Mathwin *et al.* 2021; Wassens 2005). The initial delivery of e-water began in the spring months (September-November) to mimic natural pre river regulation flows (Pyke 2002). All wetlands were pumped from dry to avoid pressure from predating and competing species (Beranek *et al.* 2021). The novel targeted water regime implemented in this project involves smaller consecutive top-ups to ensure fringing vegetation is inundated at each wetland over an extended portion of time. While conventional water delivery allows water height to slowly draw down, once water levels drop approximately 30cm within this targeted approach, a top-up is triggered to inundate the fringing vegetation as a habitat and food resource for *L. raniformis* across each wetland (Figure 2). In some instances, float valves were used where conditions were permitted to automate the process. This trigger point of 30cm was chosen to ensure that sufficient water could inundate fringing vegetation and encourage aquatic vegetation growth, which many frog and tadpole species rely on (Hoffman 2018). Following the start of each water delivery regime, *L. raniformis* breeding and recruitment were monitored at regular intervals. This report presents the outcomes of these investigations and identifies recommendations for future e-water delivery.

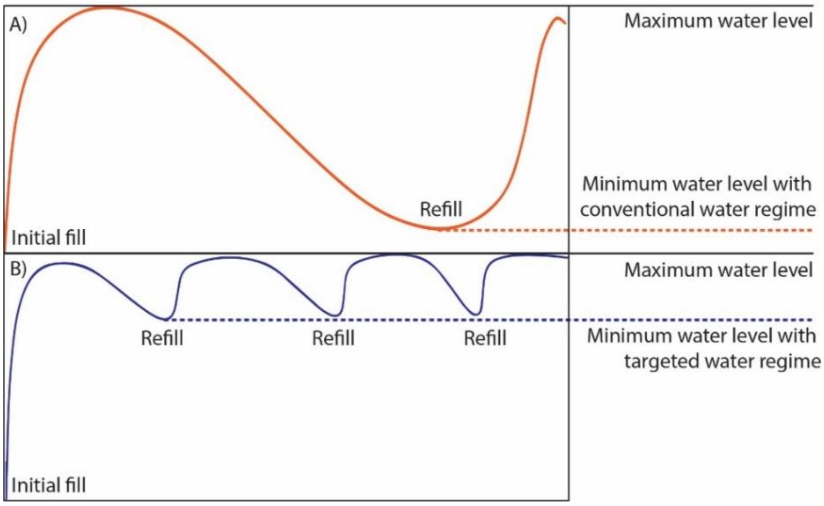


Figure 2. Diagram showing the difference in water level at temporary wetlands receiving a conventional regime (A) vs target (B).

1.1 Project objectives

This project aimed to support a higher level of southern bell frog *(Litoria raniformis)* recruitment by delivering a targeted e-water regime to temporary wetlands in the Riverland. It is hypothesised that the targeted water regime will induce a greater success in southern bell frog recruitment as this delivery system provides optimal breeding and development habitat and food for tadpoles and juvenile frogs.

The main objectives of the project were to:

- Deliver a targeted water regime to 5 wetlands in the SAMDB
- Compare *L. raniformis* recruitment success in wetlands with a targeted water regime to those which had the conventional e-water delivery method
- Provide recommendations for future e-water delivery with a focus on supporting a higher level of *L. raniformis* recruitment

Figure 3. Southern bell frog *(Litoria raniformis)*

2 Methods

2.1 Site selection

Seven wetlands were selected as sites known to be previously occupied by *L. raniformis* and contained suitable habitats. Suitable habitat for *L. raniformis* is defined by a wetland containing dense sections of lignum and, once inundated, supports the growth of aquatic and fringing vegetation, including nardoo (*Marsilea drummondii*), milfoil (*Myriophyllum tuberculatum*), and sedge sp (*Carex)* (Robinson 2019 unpublished; Hoffman 2018) (Figure 4). All wetlands chosen for this project fell within the South Australian River Murray corridor, with the furthest site located only 500 metres from the main river channel. The wetlands formed two clusters: Overland Corner and Morgan (Figure 5). The Overland Corner cluster included 3 of the following wetlands: Akuna, Overland Corner (main basins), and Old Parcoola (main basin), all are located in the lock 3 reach stretching 85km along the Murray River. The Morgan cluster included the remaining 4 wetlands: Morgan East, Morgan CP (South temporary basin), and Hogwash bend (central basin), all are located in the lock 2 reach stretching 88km along the Murray River.

Figure 4. Overland Corner wetland showing suitable *L. raniformis* habitat

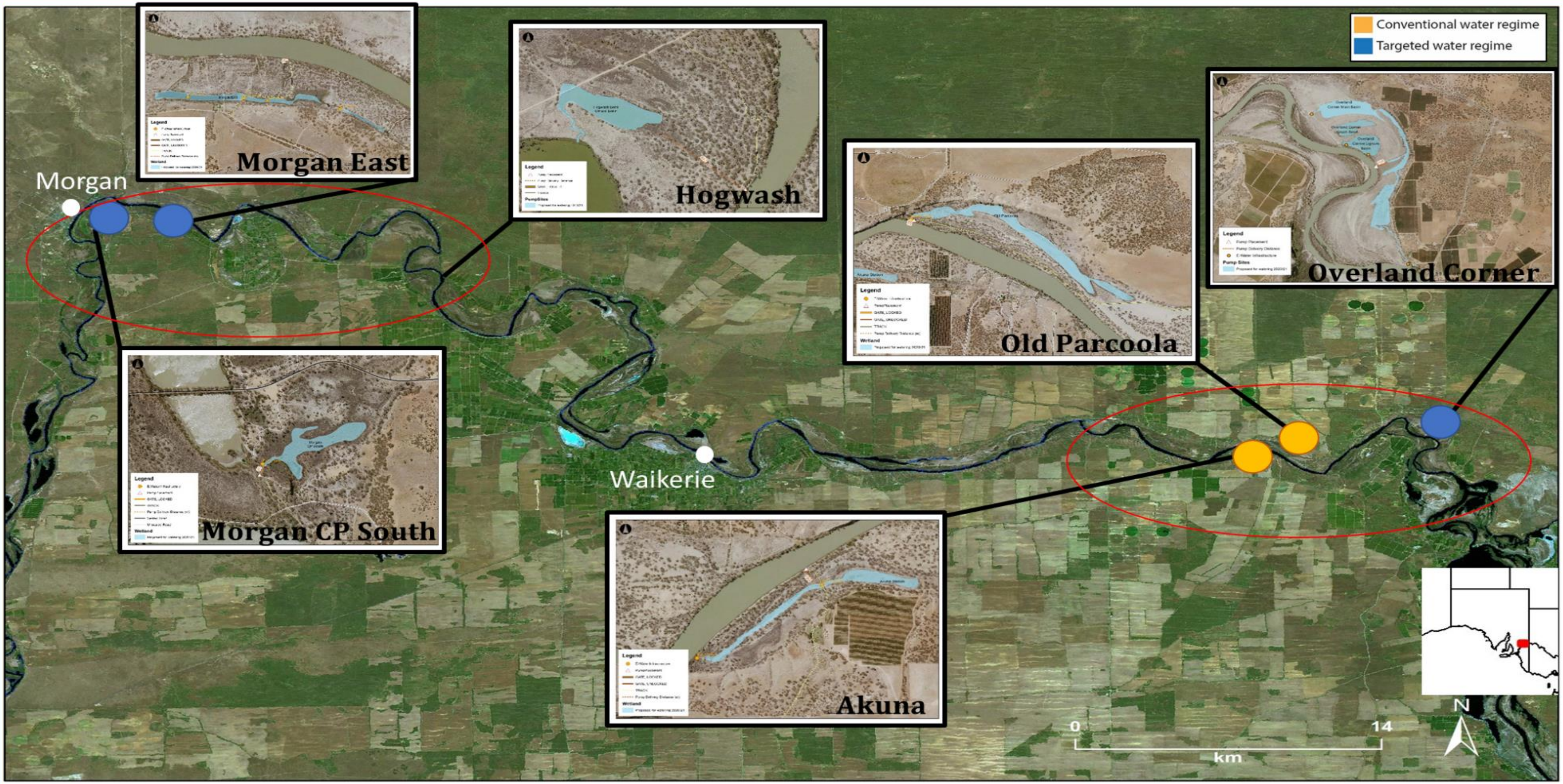


Figure 5. *L. raniformis* site selection map Riverland, SA.

2.1.1 Site descriptions

The Overland Corner wetland complex is located adjacent to the River Murray, 5kilometres north of Kingston-on-Murray. The wetland complex consists of two temporary wetlands that cover 78 ha. The temporary wetlands include the main basins (56 ha) and lignum basins (22 ha). E-water was delivered to the main basins for this project which has two sections of wetland that are structured differently. Half of the basin contains a deep open water lagoon with minimal vegetation and the other half consists of dense lignum (Figure 6). Once inundated, the dense lignum section of the main basins encourages the growth of the aquatic and fringing vegetation including nardoo, milfoil, and sedge *spp* (Figure 7).

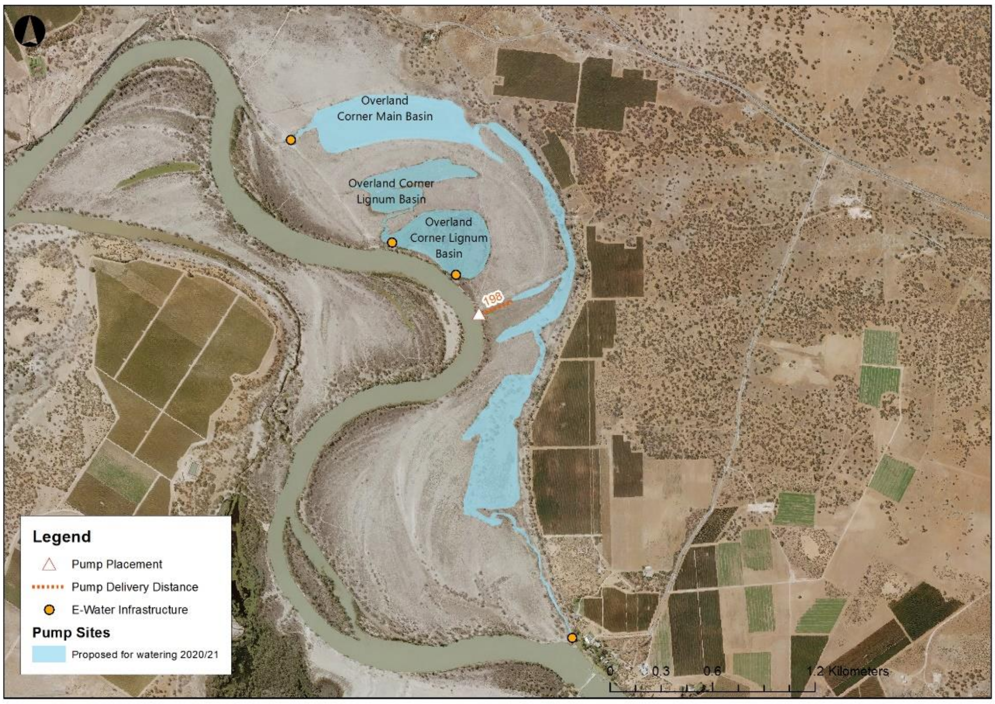


Figure 6: Overland Corner inundation map

Figure 7. Overland Corner main basin in dense lignum section of wetland 2021

Morgan East wetland is located adjacent to the River Murray, 3km east of Morgan township. The temporary wetland has a total area of 8.5 ha and is divided into five sections connected through pipe culvert e-water infrastructure under dirt access roads (Figure 8). Each structure is fitted with controlling mechanisms that prevent water from draining through the bottom end of the wetland system and allow it to be held up in each section. E-water was delivered to the entire wetland and controlled using the existing infrastructure during this project. Most sections of the wetland are relatively deep and, once inundated, provide a range of aquatic and fringing vegetation (Figure 9). However, only one section of the wetland is low-lying and contains dense lignum (closest to the pump).

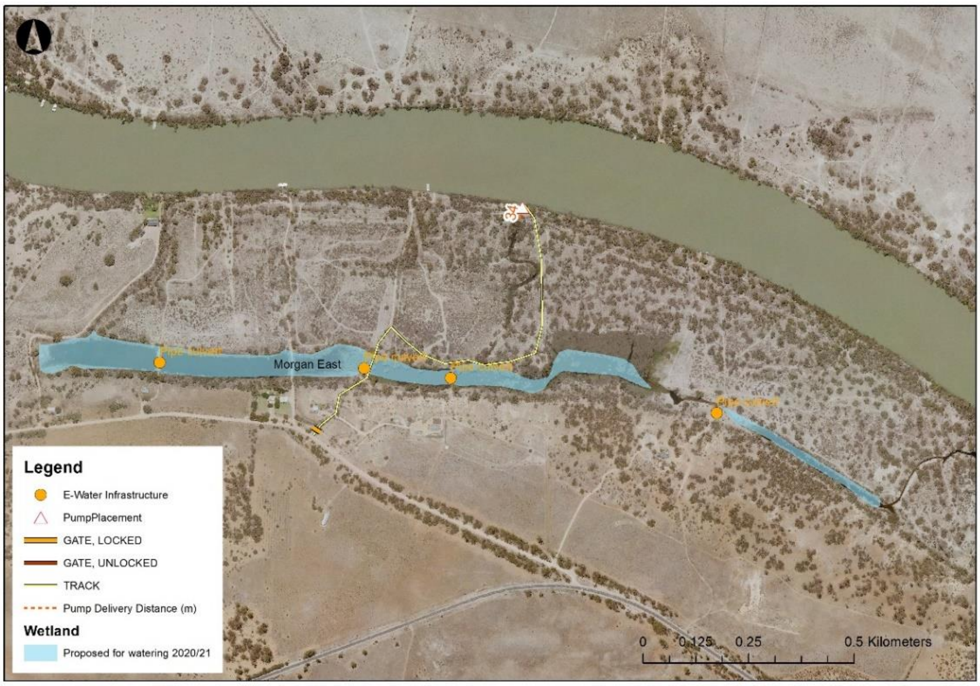


Figure 8. Morgan East wetland inundation map

Figure 9. Morgan East wetland in section of dense lignum and nardoo

Hogwash Bend wetland complex is located adjacent to the River Murray, 15km east of Morgan. The wetland complex consists of four temporary wetlands known as Hogwash East, South, North, and central basin covering 40.3 ha. E-water was delivered to the central basin, identified as a low-lying lignum basin (Figure 10). Compared to other temporary wetlands within the Hogwash complex, the lignum basin is small and contains dense lignum (Figure 11). Similar to the other wetlands chosen, Hogwash central basin offers a wide range of aquatic and fringing vegetation once inundated.

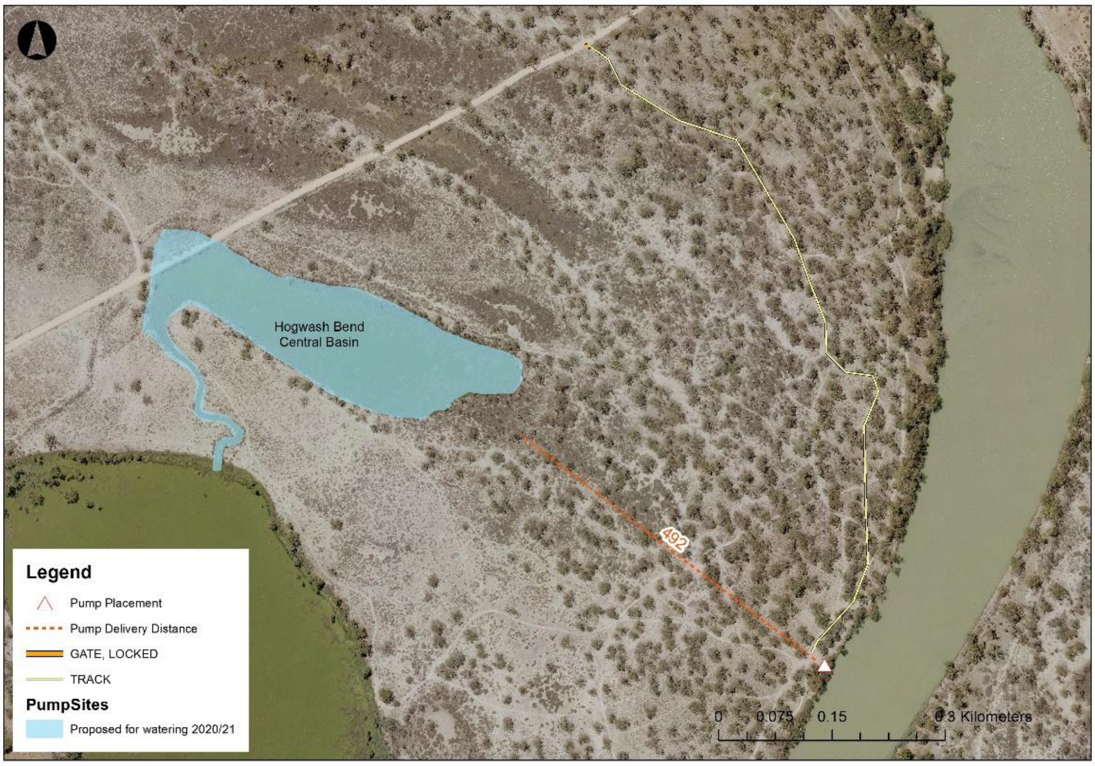


Figure 10. Hogwash Bend central basin inundation map

Figure 11. Hogwash Bend central lignum basin

Morgan Conservation Park Wetland Complex is located adjacent to the River Murray, 1km from Morgan township and on the river's east bank. The wetland complex consists of several permanent and temporary wetlands covering 60 ha. The permanent south and north lagoons cover an area of 45 ha, whilst the northern and southern temporary basins cover the remaining 15 ha. E-water was delivered to 6 ha of the Morgan CP south temporary basin, also known as the lignum basin (Figure 12). This basin is densely vegetated with lignum and has low-lying sections that offer a range of aquatic and fringing vegetation (Figure 13).

Figure 12. Morgan CP south temporary basin inundation map

Figure 13. Morgan CP South temporary basin

Akuna Wetland is located adjacent to the River Murray and is 12km west of Kingston on the Murray. The temporary wetland covers 11 ha and consists of a low-lying lignum basin and an open main basin connected through a pipe culvert road structure fitted with a regulating control mechanism. E-water was delivered to the entire wetland during this project (Figure 14). The lignum basin section of the wetland is low-lying and is covered with dense lignum. While the main basin does not contain dense lignum, similar to that of the lignum basin, it still offers a range of aquatic and fringing vegetation once inundated (Figure 15).

Figure 14. Akuna Wetland inundation map

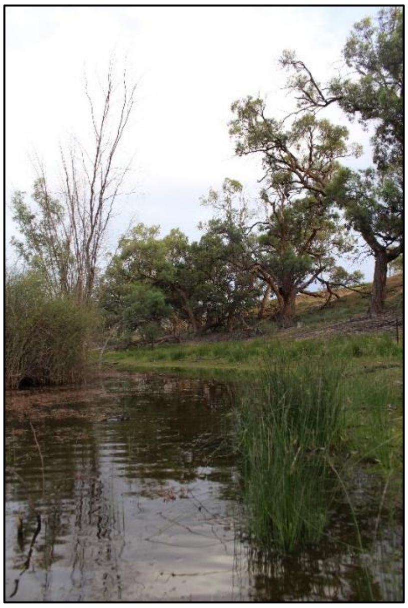


Figure 15. Akuna Wetland open main basin

Old Parcoola is located adjacent to the River Murray and is 8km northwest of Kingston-on-Murray. The Old Parcoola wetland complex consists of a few temporary wetlands such as the main basin and two flood runners covering 42 ha. E-water was delivered to the main basin for this project, and the main basin contains a large open water section and two smaller dense lignum sections (Figure 16 & Figure 17). The entire wetland offers a range of emergent, aquatic, and fringing vegetation, similar to the other wetlands described.

Figure 16. Old Parcoola inundation map

Figure 17. Old Parcoola dense lignum section of wetland

2.2 Water regime

For the purpose of this project, all wetlands were considered terminal wetlands, so pumping was used as an e-water delivery method; only at a higher flow band would they be considered flow-through systems. Two wetlands (Old Parcoola and Akuna) received the conventional water regime. In comparison, the targeted water regime was implemented at 4 wetlands: Morgan East, Morgan Conservation Park (south pump site), Overland Corner (main basin), and Hogwash (central basin) (Table 2). A float valve was placed at 2 wetlands to simulate a targeted water regime whilst manual top-ups were conducted at the other 2 target wetlands. Throughout the project, targeted wetlands received at least 3 or more tops ups in water level. The 2 wetlands receiving a conventional water regime were pumped manually with one initial fill and once top up once levels had dropped considerably. The size and configuration of pumps varied across sites depending on the volume required and existing pumping infrastructure (see Table 1). Where feasible, a float valve arrangement was installed to automate pumping when levels receded by approximately 30-40cm. At sites where this was not feasible, pumps were operated manually. The rate at which water levels were drawn down by evaporation and seepage varied across sites.

All wetlands, except for Akuna, were dry when pumping began. This method can reduce the likelihood of fish and crustaceans entering the system, which has been known to impact tadpole abundances (Robinson 2019 unpublished). Akuna wetland exhibited an earlier fill in the autumn (April 2020) to prevent the decline in the health of long-lived vegetation and therefore retained water before re-filling in September 2020. E-water was initially delivered to all other wetlands between August-December 2020. The timing of initial water delivery was based on the understanding that *L. raniformis* start calling in mid-spring (October) and continue through to mid-summer (February) (Wassens *et al.* 2008; Pyke 2002). The delivery of e-water to temporary wetlands between these months gives *L. raniformis* a better chance of successfully recruiting over a four-month time frame before the autumn and winter months (Wassens *et al.* 2008b). The top-up delivery of e-water continued until as late as March to ensure enough water was available during recruitment.

Table 1. E-water delivery information for temporary wetlands

Wetland	Type of pump	Top-up Delivery mechanism	Delivery length (m)	No. of top ups	Total volume delivered (ML)
Overland Corner	Electric 400mm	Manual top up	198	3	1446
Hogwash Bend	Relocatable diesel 150mm	Float valve	492	3+	60.6
Morgan CP	Relocatable diesel 150mm	Float valve	60	3+	112.9
Morgan East	Relocatable diesel 200mm	Manual top up	34	3+	197.4
Old Parcoola	Relocatable diesel 300mm	Manual top up	28	1	572.4
Akuna	Relocatable diesel 300mm	Manual top up	144	1	74.8

Table 2. Temporary wetlands surveyed between September 2020 and February 2021

Wetland	**Approximate Commence to flow (ML/day)**	**Easting**	**Northing**	**Water regime**	**Area (ha)**	**Sites sampled**	**Initial fill**	**Round 1 survey**	**Round 2 survey**	**Round 3 survey**
Akuna	30,000	430547	6218107	Conventional	11	3	15 Apr 2020 22 Sep 2020	12 Oct 2020	9 Nov 2020	3 Dec 2020
Old Parcoola	33,000	432838	6218596	Conventional	22	3	20 Sep 2020	19 Oct 2020	18 Nov 20	16 Dec 2020
Morgan CP South	35000-40,000	378674	6232984	Target	6	2	03 Dec 2020	07-Jan-21	27 Jan 2021	25 Feb 2021
Morgan East	40,000	380684	6233823	Target	7	2	27 Oct 2020	21 Nov 2020	30 Dec 2020	28 Jan 2021
Overland Corner	26,000	440685	6219517	Target	56	3	28 Aug 2020	08 Oct 2020	27 Oct 2020	01 Dec-2020
Hogwash	50,000	393427	6229182	Target	4.4	2	04 Nov 2020	17 Dec 2020	25 Nov 2020	14 Jan 2021

2.3 Sampling method

Breeding and recruitment were monitored using aural surveys, spotlight, and tadpole surveys, targeting different life stages or behaviours of *L. raniformis*. Aural surveys were used to target the calling abundance of male frogs (a male frog's intent to breed with a female), spotlight surveys were used to target female and juvenile frogs, and tadpole surveys were used to record the progression of tadpole development.

Monitoring was performed in 3 rounds at each wetland to observe the progress of tadpole development. Each survey took place 3 to 4 weeks following the previous survey, i.e., round 1 surveys were conducted 3-4 weeks after initial fill, round 2 surveys took place 3-4 weeks after round 1, etc. During each round, all surveys were conducted at 2-3 sites within each wetland based on the size of the wetland (2 at smaller: 0-10 hectares and 3 at larger: 10-60 hectares) (Table 2). The monitoring sites at each wetland were primarily situated in a suitable *L. raniformis* habitat (i.e. dense vegetation) and were spaced approximately 500 meters apart within fringing vegetation.

2.4 Call detection and visual spotlight survey

Nocturnal aural (sound) and visual surveys were conducted 3 times at each wetland at night between approximately 6-10pm (commencing 30 minutes after sunset at the earliest). Following the methodology outlined by Tucker (2004), 5-minute aural recordings were used to identify species present and an approximation of the abundance of male frogs calling. Sound recordings were captured using FrogSpotter smartphone app (the citizen science component of FrogWatchSA) and uploaded to make the call recordings available to the general public (available at frogwatchsa.com.au).

Alongside each 5-minute aural recording, a 10-minute visual spotlight search was conducted and covered approximately 100 meters of the wetland edge (Wassens 2008). The total number of frogs observed were recorded across all species, and *L. raniformis* individuals were classified as either an adult or juvenile based on a visual approximation of size and visual presence of a tail stump. This was undertaken observationally, without handling.

2.5 Tadpole survey

Two single-wing fyke nets (specialised fishnets) were set in or around fringing or emergent vegetation at each survey site to capture tadpoles and other aquatic fauna in conjunction with each nocturnal survey. Nets were set pre-dusk (4-7pm) and left overnight to be retrieved the following morning between approximately 6-11 am (starting at sunrise) for an average total set time of 19 hours depending on weather conditions (nets were set for less time during extreme heat). Nets were cleaned in a diluted bleach solution and air-dried in the sun for at least a day before re-use to avoid potentially transferring pathogens between sites.

For every net, the tadpole genus (identified using Antis 2007), Gosner's stage of development (Gosner 1960) (Table 3), and length (mouth to tail tip (millimetres) were recorded for the first 15 individuals of a species as a representative sample of each net. Following the first 15 individuals, only the abundance of each species is recorded. Pairing this method with aural and spotlight surveys tracks the progression of tadpole development over the study period, and specifically successful *L. raniformis* recruitment by identifying juveniles through all stages of metamorphosis. In addition to tadpole abundance and development, all other aquatic fauna species caught; specifically fish and crustaceans, were identified and counted.

Figure 18. Old Parcoola tadpole survey round 1 October 2020

Table 3. Gosner's stage of tadpole development. Major stages of development are bolded and highlighted in grey (adapted from Gosner 1960)

Stage	Defining character
20-23	Cornea becomes transparent
24	External gills atrophy
25	Transition to feeding and free swimming tadpole
26-30	***Development of small hind buds***
31-37	Buds transform into toes
38-39	***Hind leg fully forms and toes become webbed***
~~40~~	~~Cloacal tail piece starts to shrink between fully formed hind legs~~
41	cloacal tailpiece lost and forelimb buds start to form
42	***Forelimbs emerge***
43-44	Widening of mouth
45	Tail stub present
46	Tail stub gone
47-50	***Tadpole becomes juvenile frog***

At both the night call survey and morning tadpole survey, surface water quality was taken to monitor water quality at e-water sites during the project. A handheld multimeter (U-50 series Multi Water Quality Checker) was used to assess water quality during the surveys. Parameters included:

- Salinity (represented as electrical conductivity).
- Temperature (degrees Celsius).
- pH.
- Dissolved oxygen (milligrams per litre).
- Turbidity (nephelometric turbidity units).

2.6 Data analysis

The highest calling abundance of male *L. raniformis* was recorded at each wetland during each survey round. As counting frog abundance is difficult when present in high numbers, abundance scores were used (Table 4) (Hoffman 2017 & 2018; Wassens 2005). Wetlands, where no *L. raniformis* were heard calling, have been omitted from the results.

Table 4. Highest calling abundances categories for *L. raniformis*

One=1	**Few=2-9**	**Many=10-50**	**Full chorus= 50>**
Small	Moderate	High	Highest

The total abundances of *L. raniformis* tadpoles were recorded for each wetland and compared between wetlands and water regimes. In addition to total abundances, average tadpoles per net were calculated using the average of total catch per net across the 3 survey rounds along with the standard error to allow for wetland comparison.

The proportion of *L. raniformis* tadpoles caught during each survey round was graphed and compared between target and conventionally watered wetlands. The proportion was calculated by dividing the total number of tadpoles caught in each round by the number of wetlands receiving the different water regimes (five targeted and two conventional).

The combination of spotlight visual data and the fyke net data enabled the tracking of the development of *L. raniformis* tadpoles into juvenile frogs. The development stages of *L. raniformis* were compared over the three surveys between target wetlands and conventionally watered wetlands.

3 Results

3.1 Call detection

The highest calling abundance of *L. raniformis* was detected at a majority of the wetlands during the first survey round, except Old Parcoola where no *L. raniformis* were heard calling (Figure 19). The highest calling abundance of 10-50 *L. raniformis* was recorded at Morgan East 5 weeks after the initial pumping in October 2021. In rounds 2 and 3 *L. raniformis* were only heard calling in moderate numbers at three target sites (Figure 19).

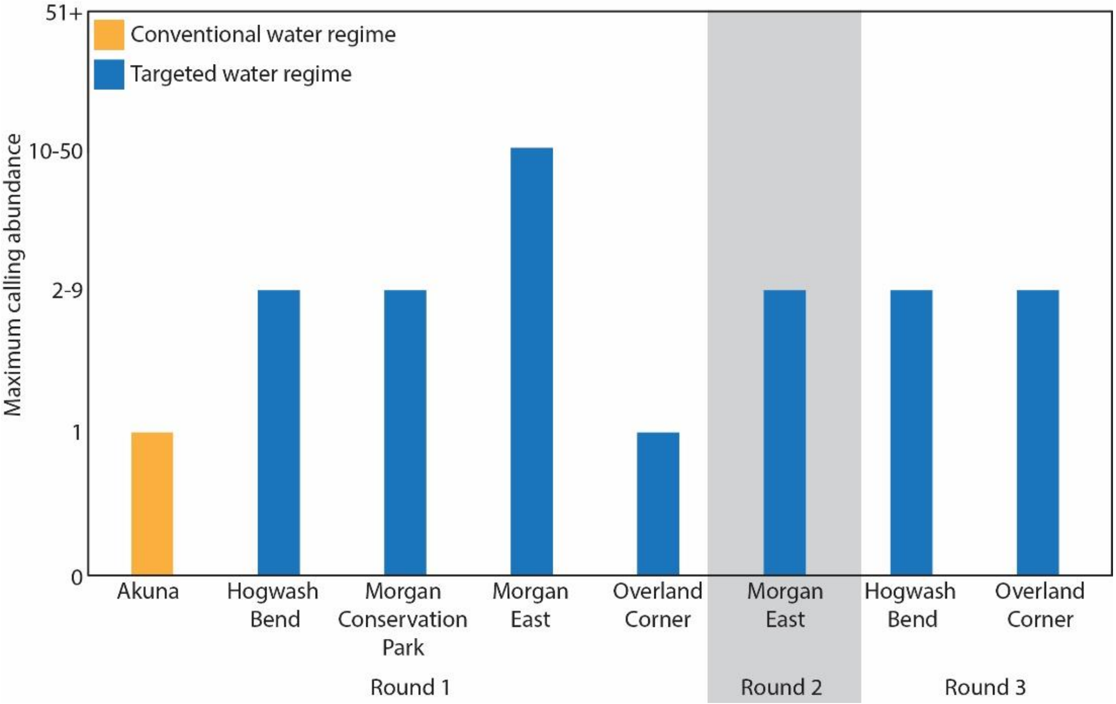
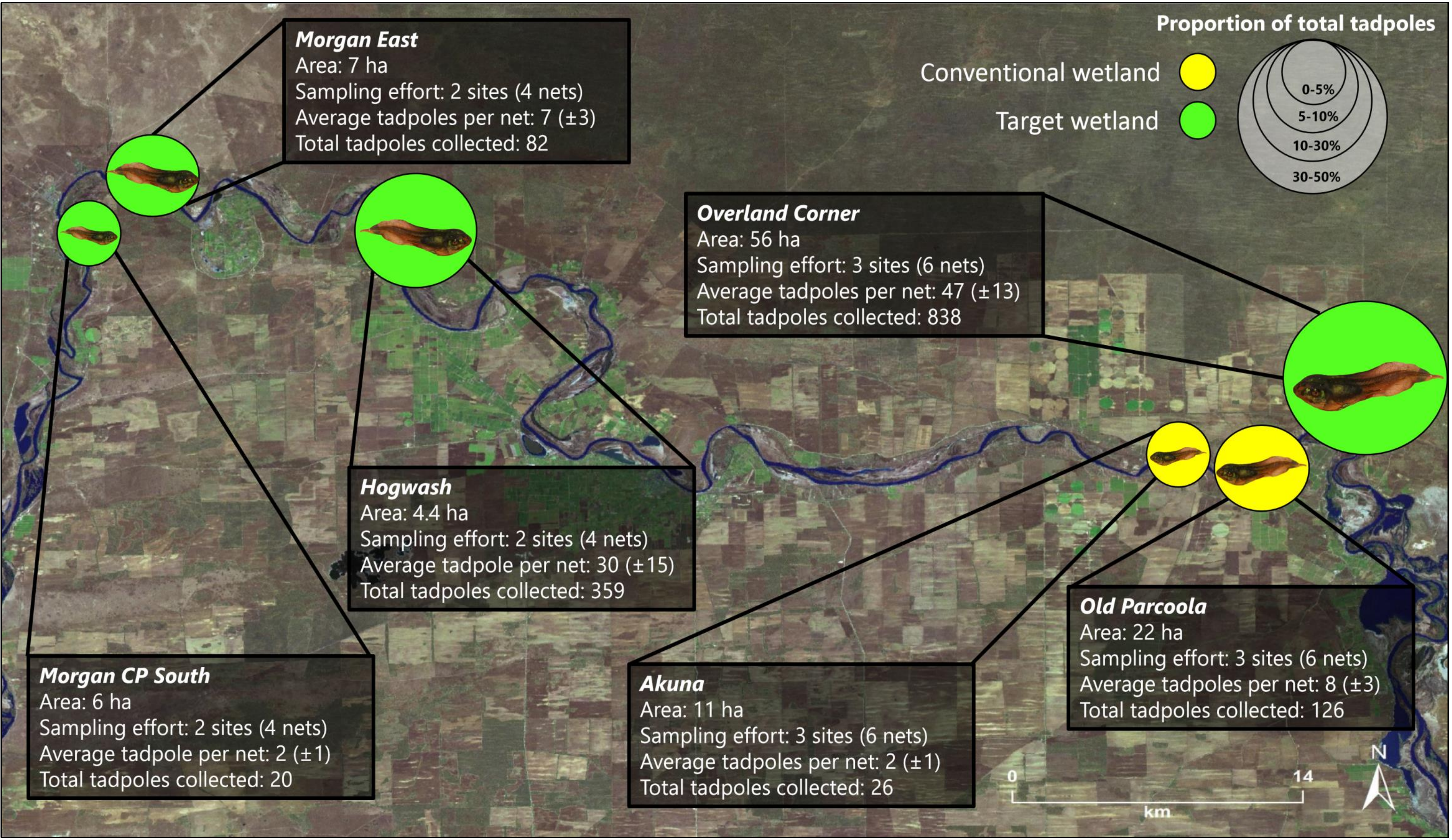


Figure 19. Highest calling abundance of *L. raniformis* at all temporary wetlands

3.2 Tadpole abundance

A total of 1,450 *L. raniformis* tadpoles (at all life stages) were captured across all wetlands over the 3 survey rounds, 60% of which were captured at Overland Corner and a further 30% at Hogwash Bend (Figure 20). Both wetlands received a targeted water regime and had the highest number, and proportion of tadpole captures compared to wetlands receiving the conventional water regime. Overland Corner had the highest total abundance of *L. raniformis* tadpoles (838 total) along with the highest average tadpoles caught per net (47 per net) across the 3 survey rounds. Hogwash had the second-highest abundance of 359 tadpoles caught in total and an average of 30 tadpoles per net across the 3 surveys. The average total of tadpoles caught at targeted wetlands (324) was 4 times more than the average of conventional wetlands (75). Both the conventional watered sites had a much smaller total abundance of tadpoles and average catch per net with a total of 126 caught at Old Parcoola and an average of 8 per net, and a total of 26 tadpoles caught at Akuna across the 3 surveys with an average of 2 caught per net.



Morgan East
Area: 7 ha
Sampling effort: 2 sites (4 nets)
Average tadpoles per net: 7 (±3)
Total tadpoles collected: 82

Overland Corner
Area: 56 ha
Sampling effort: 3 sites (6 nets)
Average tadpoles per net: 47 (±13)
Total tadpoles collected: 838

Hogwash
Area: 4.4 ha
Sampling effort: 2 sites (4 nets)
Average tadpole per net: 30 (±15)
Total tadpoles collected: 359

Morgan CP South
Area: 6 ha
Sampling effort: 2 sites (4 nets)
Average tadpole per net: 2 (±1)
Total tadpoles collected: 20

Akuna
Area: 11 ha
Sampling effort: 3 sites (6 nets)
Average tadpoles per net: 2 (±1)
Total tadpoles collected: 26

Old Parcoola
Area: 22 ha
Sampling effort: 3 sites (6 nets)
Average tadpoles per net: 8 (±3)
Total tadpoles collected: 126

Figure 20. Map of *L. raniformis* tadpole abundance across temporary Riverland wetlands

Other tadpole species that were caught and recorded during the surveys included: spotted marsh frog (*Limnodynastes tasmaniensis),* banjo frog *(Linodynastes dumerilii),* long-thumbed frog (*Limnodynastes fletcheri),* Peron's tree frog (*Litoria peronii),* Sudell's frog (*Neobatrachus sudelli*) and the eastern-sign bearing froglet *(Crinia parinsignifera).* For the purpose of this project, the *Limnodynastes spp.* were grouped as one as they were difficult to differentiate at tadpole stages. A total of 86,604 tadpoles were caught across the 3 surveys rounds at all wetlands. Of those, the most abundant were the *Limnodynastes spp*., which made up 83% of the total catch; a further 10% were *L. peronii* and 2% *L. raniformis.* Hogwash and Overland Corner (both target sites) had the highest total catch of all tadpole species across the 3 rounds, with totals ranging from 28,978 to 29,531 individuals.

Fish, crustaceans, and reptiles were also caught and recorded during the survey rounds and were included as bi-catch. Of the aquatic fauna species caught, fish, crustaceans, and reptiles made up 11% of the total catch with amphibians the most dominant at 89%. Morgan East had the highest proportion of other aquatic fauna species caught (60%) and the lowest proportion of amphibians (40%) compared to Overland Corner, which only had 1% of aquatic fauna and 99% amphibians. Of the total fish caught, 95% were native and 5% invasive; the most commonly caught species were carp gudgeon, goldfish and common carp.

3.3 Proportion of *L. raniformis* tadpole catch Target vs Conventional

Round one surveys revealed similar numbers of *L. raniformis* tadpoles (20-30% of all tadpoles caught across the surveys) collected, regardless of water regime (Figure 21). At target wetlands, the proportion of *L. raniformis* total catch of tadpoles peaked at 50% in round 2 and dropped to 20% in round 3. Where a conventional water regime was delivered, the proportion of *L. raniformis* tadpoles dropped in round 2 to 2% of the total catch and further in round 3 to 0%.

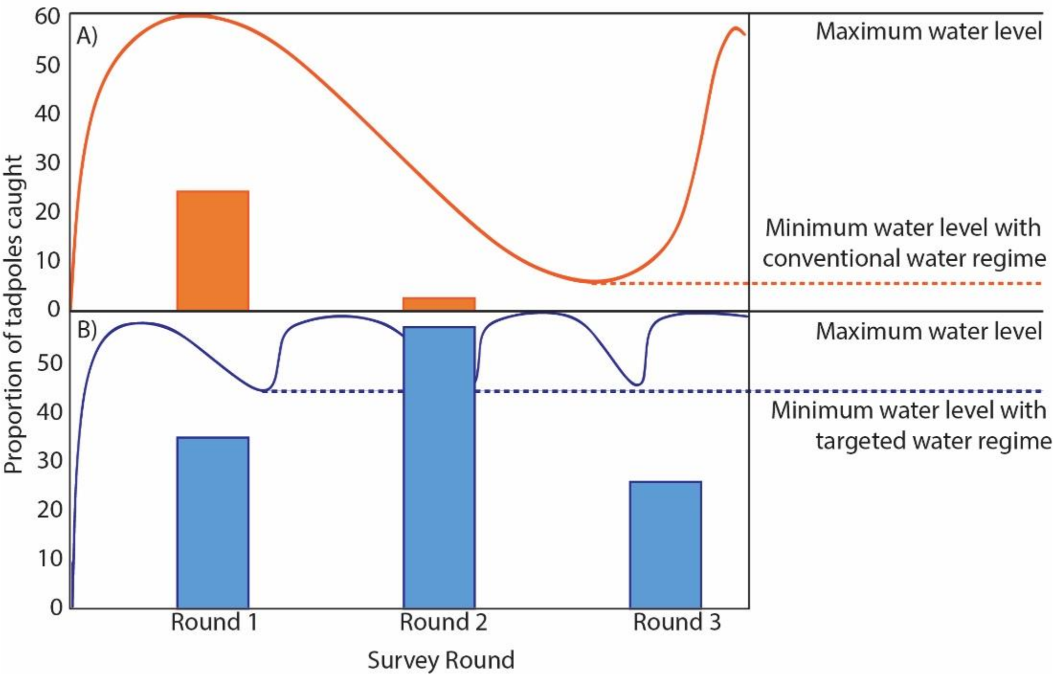


Figure 21. Proportion of *L. raniformis* tadpoles caught during each survey round at conventional (A) vs target (B) wetland

3.4 Southern bell frog recruitment

During the first survey round, the presence of small tadpoles was identified at all temporary wetlands, with the development stages in the range of 20 to 25 observed. In round 2, tadpole development progression was evident at targeted water regime wetlands as most tadpoles collected were already showing signs of hind legs (stages 38 to 39). In the final round of surveys, tadpoles in late developmental stages (presence of forelimbs, stages 42 to 43) were identified, and juvenile *L. raniformis* (stage 47 to 50) were detected at most of the target wetlands, including Overland Corner, Morgan East, and Hogwash. While tadpole development appears to have progressed across the survey rounds at the targeted wetlands, those wetlands with conventional water regimes exhibited limited developmental progression, with the highest tadpole development stage observed being 41 and no juvenile frogs observed (Figure 22).

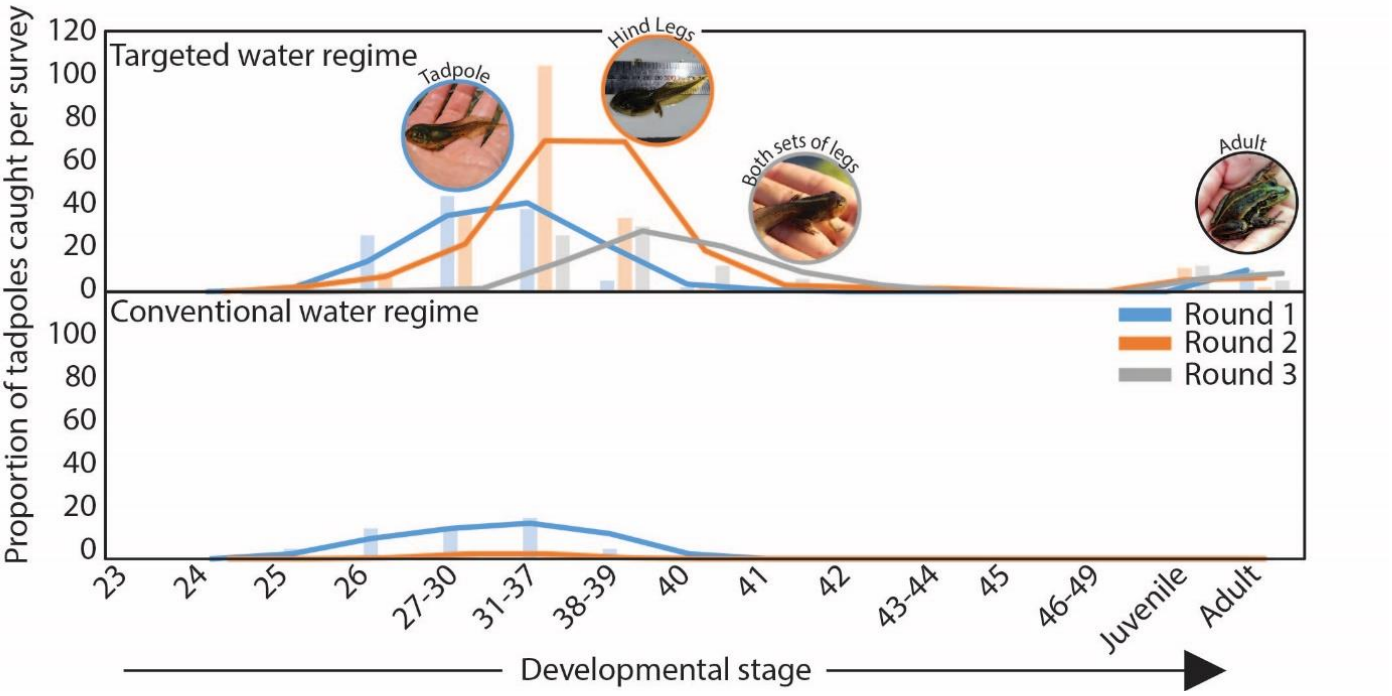


Figure 22. Proportion of *L. raniformis* caught per developmental stage over sampling round 1 (blue), round 2 (orange), and round 3 (grey). The top panel displays the targeted water regime while the bottom panel displays the conventional watering regime. The solid lines display the 2-point moving average.

3.5 Surface water quality

Surface water quality was measured as a part of this project to monitor water quality parameters at wetlands receiving e-water during *L. raniformis* surveys. Surface water parameters were within expected ranges, and no trends were observed between water quality and *L. raniformis* recruitment (Appendix A). Variable DO levels are not uncommon for ephemeral wetlands (Podrabsky *et al.* 1997).

4 Discussion

Preliminary analysis of frog and tadpole data showed that e-water, when delivered in a targeted manner, can play an essential role in triggering natural breeding cues for *L. raniformis* and promote successful recruitment. Regardless of wetland size, calling abundances were generally greater at sites receiving regular top-ups (targeted water regime). The higher calling abundance at sites receiving the target regime is likely because frog breeding is triggered by wetting events as watering regimes may provide newly inundated habitats for successful recruitment. Similarly, the overall successful recruitment of *L. raniformis,* in temporary wetlands was only found at sites receiving a targeted water regime, suggesting this response was to the availability of suitable water for a prolonged period, enough for tadpoles to fully develop and a lack of competitive/predatory species. The results of this project emphasize the need to design and implement delivery regimes targeted towards ecological outcomes such as frog recruitment success during the annual e-water planning process. Conventional e-water delivery regimes fail to provide the habitat suitability needed for species like *L. ranifomis*. Without incorporating the ecology of native species into management plans, wetland species are much more vulnerable to current and future changes.

4.1 Southern bell frog breeding and recruitment

Aural call surveys indicated that *L. raniformis* show a rapid breeding response to the initial delivery of e-water, regardless of water regime. However, only a few wetlands (i.e., those with a targeted water regime) maintained frog calling through each survey round. Overland Corner, Morgan East, and Hogwash – wetlands receiving targeted water regime— continued to have a significantly higher calling abundance of *L. raniformis* across the survey rounds, even 3 months after the initial wetland fill. These wetlands contained a larger area of dense inundated lignum when compared to the other wetlands. Lignum and fringing vegetation, including sedge spp., may be more attractive than reeds and samphire to adult *L. raniformis* during the initial breeding process due to the vegetation's complex structure, which provides habitat and better cover from predators. All wetlands (conventional and target) had some areas of dense lignum and fringing vegetation along with aquatic vegetation. However, those receiving the targeted water regime exhibited a larger area of suitable habitat inundated due to maintaining water levels through consecutive top-ups.

Similar to the benefits of emergent vegetation for frog breeding, the abundance of submerged aquatic vegetation at certain wetlands may have provided additional resources to juveniles and tadpoles. Overland Corner and Hogwash exhibited the highest proportion of *L. raniformis* tadpoles caught across the three survey rounds (

Figure **23**). From general observations during the surveys, both aforementioned targeted water sites had an abundance of submerged aquatic vegetation, including watermilfoil and nardoo (species known to be a food resource for tadpoles), that persisted over a long period (Figure 24). Hogwash lignum basin is very small compared to Overland Corner but is largely dominated by submerged lignum and aquatic vegetation. Overland Corner main basins have sections with adequate vegetation; however, half of the wetland is open water with little to no vegetation. Generally, the wetlands receiving a targeted water regime continued to have a higher proportion of tadpoles caught across the 3 survey rounds. Conventionally watered wetlands with receding water levels had a proportionately lower catch of *L raniformis* tadpoles over the 3 survey rounds.

Figure 23. Many *L. raniformis* tadpoles caught during round two at Hogwash

Figure 24. Submerged aquatic and fringing vegetation (nardoo and sedge) at Overland Corner

At Akuna and Old Parcoola, water levels began receding by round 2 surveys, which meant that tadpoles and frogs were drawn away from fringing vegetation and concentrated into open water with limited food resources and more susceptible to predation due to the lack of inundated habitat. Both conventional wetlands had one monitoring site located in dense lignum and by round 3, nets were unable to be set in these sections as they were no longer inundated. Therefore, both wetlands went from 3 monitoring sites to 2. Similar to round 2, tadpoles and frogs were drawn out of areas of suitable habitat into open water. Wetlands receiving the conventional water regime had shown no signs of successful *L. raniformis*, indicating that receding water levels may lead to the loss of adequate habitat and food resources to support the progress of *L. raniformis* tadpole development.

Tadpole metamorphosis for *L. raniformis* takes around 3 months and not only is an abundance of food resources required for this duration (Antis 2013; Mann *et al.* 2010), but adequate water needs to be present throughout the metamorphosis process. Without regular top-ups, wetlands that received the conventional water regime could no longer sustain water levels to support tadpole development. Wetlands receiving the targeted water regime, including Overland Corner and Hogwash, were inundated for three months. At that time, *L. raniformis* tadpoles developed into juvenile frogs by the third survey round (Figure 25). The area of suitable habitat for tadpoles and food resources was maximised by maintaining water levels.

Figure 25. Juvenile *L. raniformis* observed during the spotlight search at Hogwash round three

As *L. raniformis* tadpole numbers declined with receding water levels at conventional wetlands, frog calls ceased completely. At Akuna Wetland, *L. raniformis* were only heard calling during round 1, and while tadpoles (in small numbers) were found during the following rounds, breeding calls had ceased by the last 2 surveys. Old Parcoola was also a conventionally-watered wetland where *L. raniformis* were not heard calling in all 3 of the manual call detection surveys. Past surveys reveal that *L. raniformis* were detected at both wetlands, which is not unusual considering these wetlands are of proximity to Overland Corner (wetland known to have strong *L. raniformis* breeding response to watering). In 2019, automated sound recorders recorded *L. raniformis* calling in high abundances at this site 2-14 days following initial pumping (Robinson 2019 unpublished).

Given that the first round of manual call surveys in 2019 was conducted 4 weeks following initial pumping, the opportunity to capture recordings in the main breeding window may have been missed at some sites. Ideally, all call surveys would have taken place 2-14 days after the initial fill. However, due to time constraints, the call surveys were conducted in conjunction with the other survey methods as more of an opportunistic survey. Therefore, although *L. raniformis* were not heard calling at Old Parcoola in round one, it does not necessarily mean that they had not already initially bred in the first 2-14 days. Declines in breeding and recruitment over time may result from continuously receding water levels from conventional water regimes, making suitable habitats scarce, especially for those life states that are restricted to water (Wassens *et al.* 2008a).

4.2 Future management/recommendations

The following recommendations have been made to help guide future management regarding the delivery of e-water for *L. raniformis* recruitment. Findings from this project and past studies have been considered when making recommendations for site selection, water regime, benefits to other species and future surveys/research with a particular focus on supporting *L. raniformis* recruitment.

4.2.1 Site selection

When selecting sites to support *L. raniformis* recruitment, it is recommended that a few key factors be taken into consideration. Firstly, past call surveys should be assessed to establish where *L. raniformis* have been previously located (Robinson 2019 unpublished; Hoffman 2018). Generally, these sites will be temporary wetlands adjacent to the main river channel as these are typically used by breeding adults. Although *L. raniformis* may call at permanent water bodies, these sites may be less successful as the presence of competing and predating species may limit successful recruitment (Wassens *et al.* 2008a; Hoffman 2017).

Temporary wetlands can be categorised in many ways including, area size, depth, and vegetation communities. To support *L. raniformis* recruitment, smaller dense lignum basins have shown to be the more favourable type of wetland by providing adequate habitat for adult frogs. While large basins may provide increased water area, without dense structural habitat, successful recruitment may be limited compared to sites with large amounts of submerged and emergent vegetation. Further, although little is known about the distance travelled by this species, it is recommended to deliver e-water to temporary basins, with large amounts of structural habitat that are within proximity to one another to promote movement between them (Mathwin *et al*. 2021).

4.2.2 Water regime

The results from this project and other studies have provided strong evidence that temporary wetlands receiving e-water have a higher success rate of *L. raniformis* recruitment (Hoffman 2015, Beranek *et al.* 2020). When planning the delivery of e-water for *L. raniformis* recruitment, temporary wetlands must be initially dry rather than wet to ensure the absence of competing and predatory species such as fish and crustaceans, which can commonly enter through the pump and reproduce over time (Wassens *et al.* 2008a; Hoffman 2017).

E-water delivery should take place between September and December to capture the early spring breeding window of *L. raniformis*. Further, as spring floods would have occurred naturally in October (early spring), coinciding e-water delivery during this time will promote the natural breeding cycles of *L. raniformis* (Hoffman 2018). Additionally, this period optimises weather conditions to encourage tadpole survival. Delivering e-water outside this period could result in decreased survival as cooler or warmer weather would impact the growth of tadpoles and water evaporation rates (i.e., habitat availability).

The minimum hydro-period of wetland inundation should extend over 4 months with an implemented targeted water regime of smaller consecutive top-ups, either manually or with automated float valve systems. This regime maintains water levels to encourage aquatic vegetation growth (such as nardoo and watermilfoil), which are food sources and habitat refuges for *L. raniformis* tadpoles during the metamorphosis process (Wassens *et al.* 2008a; Robertson *et al.* 2002). Concomitantly, maintaining higher water levels has also been shown to trigger multiple breeding events by inundating fringing vegetation, which is predominantly used as a habitat for adult *L. raniformis* during the breeding process.

4.2.3 Umbrella species

E-water is delivered to temporary wetlands within the Riverland region to target several ecological values including frog recruitment, long-lived vegetation health, wetland-dependant bird species and fresh groundwater lens. These ecological parameters are taken into careful consideration during the annual e-water planning process. The hydro-period implemented at temporary wetlands must be extended over a certain period to achieve a majority of these ecological values.

Given the complex 3-month metamorphosis process of *L. raniformis,* using the umbrella species approach as a conservation tool could potentially benefit other species in temporary wetlands receiving e-water (Plan 2005; Branton & Richardson 2014). Umbrella species are selected to make conservation decisions, generally because protecting these species will indirectly and directly benefit and protect other species within the ecological niche (Roberge & Angelstam 2004; Breckheimer et al. 2014).

Past studies have shown that other common Riverland frog species exhibit a much shorter metamorphosis process compared to *L. raniformis*, and therefore a higher recruitment rate of these species may occur in wetlands with an implemented targeted e-water delivery regime (Hoffman 2018). By maintaining water levels over a four-month period, species such as *C. parinsignifera* with an 11-12 week metamorphosis process could have several successful breeding events (Hoffman 2018).

Long-lived floodplain vegetation would also benefit from the targeted e-water regime as species such as river red gum (*Eucalyptus camadulensis*), river coobah *(Acacia stenophylla),* black box *(Eucalyptus largiflorens*), and lignum *(Muehlenbeckia florulenta*) which require 3-4 months of inundation (Jensen & Walker 2017). Tree species such as river red gum require 2-4 months of inundation over the spring/summer time to grow, and water delivery needs to occur in approximately 2 out of every 4 years. Implementing the targeted *L. raniformis* e-water regime at temporary wetlands in the spring/summertime will provide ideal growing conditions for long-lived vegetation species (Jensen & Walker 2017).

4.2.4 Future surveys/research

When conducting intensive surveys to monitor the successful recruitment of *L. raniformis,* a combination of the following methods should be used concomitantly: aural call recording, spotlight search, and tadpole fyke net surveys. Using this multi-method approach has proven to be very effective in tracking the progression of *L. raniformis* breeding and metamorphosis as each method targets one or more developmental stage (including adult and juvenile stages) and together can be used to track breeding and recruitment success (Robinson 2019 unpublished; Hoffman 2017; Wassens 2008a). Aural call and spotlight surveys should be conducted approximately 2-14 days following initial pumping to identify both male and female frogs during the main breeding window of *L. raniformis.* A further 4 weeks later, fyke net and spotlight surveys should be conducted to allow time for tadpole development.

Environmental factors that need to be considered during monitoring include weather conditions and surface water quality. For instance, low surface water dissolved oxygen can stress tadpoles through a lack of oxygen in the water. Collection during periods of low dissolved oxygen compounds additional stress potentially, causing an increased risk of mortality. Extremely hot weather (which is commonly experienced in the Riverland) contributes to a higher rate of photosynthesis during the day and increased respiration at night, decreasing dissolved oxygen levels overnight. As the nets are set the day prior, tadpoles can move into the net but are captured overnight and therefore are unable to move towards more oxygenated water. Therefore, it is strongly discouraged to conduct surveys during periods of hot weather when concerning the survival of tadpoles.

While the information provided in this project has been critical in informing e-water delivery programs, future research could investigate the movement of *L. raniformis* between temporary and permanent water bodies. This information would contribute to a better understanding of species behaviour during different seasons and may support the idea that watering wetlands in clusters to encourage movement. Further investigation into the link between calling abundances and specific water levels could also be carried out through the deployment of automated sound recorders and 4G trail cameras to capture daily calling abundances and track water levels. The information gained through this investigation would expand on the existing knowledge of *L. raniformis* calling abundances in relation to e-water delivery and receding water levels. Photo points set up at each monitoring site could also capture the different vegetation communities that *L. raniformis* rely on during the breeding process and tadpole metamorphosis.

4.3 Conclusion

This study and other recent studies have demonstrated that the delivery of e-water via pumping can support the successful recruitment of many frog species (particularly *L. raniformis)* (Robinson 2019 unpublished; Hoffman 2018; Spencer & Wassens 2009). The findings of this project have directly assisted in adaptive management strategies providing valuable information regarding the timing and delivery of e-water to temporary wetlands in the Riverland region. Temporary wetlands located along the River Murray corridor, with a complex, diverse vegetation structure, and with a targeted e-water delivery regime, exhibited a much higher rate of *L. raniformis* recruitment. These wetlands were initially pumped from dry to exclude predating and competing species, such as fish and crustaceans. Using the targeted approach, maintaining water levels over four months meant that fringing, aquatic, and emergent vegetation were inundated for a more extended period, thus providing suitable habitat and food resources during initial *L raniformis* breeding and tadpole metamorphosis. Using this e-water tool to protect umbrella species, in this case here, *L. raniformis* will aid in the overall health and resilience of temporary wetland ecosystems.

5 Appendices

5.1 Appendix A

Table 5. Surface water results (Mean ± Standard deviation) for each parameter during the three survey rounds at each temporary wetland. * represent values that lie beyond threshold values.

Wetland	Survey	EC(μS/cm)	Turbidity	Temperature	pH	DO
Akuna	1	348.6 (± 43.1)	N/A	21.29 (± 2.3)	8.16 (± 0.4)	9.98 (± 2.27)
	2	376.67 (± 19.7)	8.58 (± 4.5)	24.33 (± 2.3)	7.48 (± 3.4)	5.82 (± 1.7)
	3	469.67 (± 13.2)	12.93 (± 7.7)	24.29 (± 2.8)	7.57 (± 0.5)	6.94 (± 2.1)
Hogwash	1	355 (± 15.6)	29.95 (± 10.3)	28.69 (± 0.5)	7.7 (± 0.1)	2.77 (± 0.4)*
	2	397.33 (± 39.5)	147.33 (± 14.2)	21.76 (± 3.4)	6.60 (± 0.1)	6 (± 2)
	3	460.6 (± 23.6)	105.96 (± 88.5)	21.54 (± 3)	6.75 (± 0.1)	6.02 (± 2.8)
Morgan CP	1	503 (± 417.9)	15.6 (± 10.9)	23.54 (± 1.7)	6.70 (± 0.3)	6.61 (± 2.8)
	2	239 (± 39.1)	48.18 (± 36.4)	22.33 (± 1.7)	6.73 (± 0.1)	1.41 (± 1.4)*
	3	327.17 (± 100.2)	24.2 (± 31)	19.91 (± 1.8)	6.59 (± 0.1)	2.04 (± 1)*
Morgan East	1	337.17 (± 99.9)	12.27 (± 9)	26.02 (± 2.2)	6.63 (± 0.5)	5.95 (± 1.9)
	2	406.33 (± 401.8)	9.8 (± 1.5)	23.1 (± 0.9)	7.03 (± 0.5)	6.31 (± 1)
	3	342 (± 63.5)	22.77 (± 9.1)	25.92 (± 2.3)	7.64 (± 0.6)	5.64 (± 4.3)
Old Parcoola	1	317.43 (± 25.4)	45.97 (± 30.3)	20.12 (±2.9)	7.49 (± 0.4)	4.43 (± 2.4)*
	2	443.33 (± 11.4)	82.03 (± 16.4)	19.89 (± 1.4)	7.99 (± 0.1)	6.02 (± 1.9)
	3	649.6 (± 43.7)	39.1 (± 5.7)	22.95 (± 2.5)	7.97 (± 0.4)	7.47 (± 1.3)
Overland Corner	1	458.33 (± 207.5)	77.93 (± 117.9)	16 (± 1.4)	7.51 (± 0.3)	7.36 (± 2.7)
	2	555 (± 250)	73.9 (± 104.4)	17.51 (± 1.2)	7.9 (± 0.6)	6.82 (± 2.3)
	3	650.67 (± 352.9)	63.7 (± 64.9)	20.67 (± 2.1)	6.64 (± 0.3)	6.90 (± 1.8)
Wigley Reach	1	335 (± 3.7)	57.2 (± 32.1)	21.22 (± 2.5)	7.09 (± 0.3)	0.7 (± 0.6)*

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