



Transport  
for NSW

# Warrell Creek to Nambucca Heads

Giant Barred Frog Monitoring Annual Report –  
year four operational phase 2021/2022

Transport for New South Wales | April 2023 | Final report





## Document Review

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# 1. Introduction

In 2015, Transport for New South Wales, in conjunction with Acciona Ferrovia Joint Venture (AFJV), commenced the upgrade of the Pacific Highway between Warrell Creek and Nambucca Heads (WC2NH). The WC2NH project was opened to traffic in two stages:

- Stage 2a - 13.5km section from Lower Warrell Creek Bridge to Nambucca Heads opened on 18 December 2017; and
- Stage 2b - 6.25km section from the southern end of the project to the Lower Warrell Creek bridge opened in late June 2018.

Approvals for the WC2NH upgrade required monitoring of several species and mitigation measures during the operational phase. Species monitored include koala (*Phascolarctos cinereus*), yellow-bellied glider (*Petaurus australis*), giant barred frog (*Mixophyes iteratus*), green-thighed frog (*Litoria brevipalmata*) slender marsdenia (*Marsdenia longiloba*), rusty plum (*Niemeyera whitei*) and Floyds grass (*Alexfloydia repens*). Mitigation measures monitored included green-thighed frog breeding ponds, fauna underpasses, vegetated median, and exclusion fence. Sandpiper Ecological Surveys (SES) has been contracted by Transport for NSW (TfNSW) to deliver the WC2NH operational ecological and water quality monitoring program in accordance with the Warrell Creek to Nambucca Heads Operational Ecological and Water Quality Monitoring Brief (the Brief).

The following report details the methods and results of the year four operational phase giant barred frog population monitoring. The objective of giant barred frog monitoring, as outlined in the Giant Barred Frog Management Strategy (GBFMS), is “to demonstrate through the life of the Project that mitigation has maintained or improved population sizes and habitat of giant barred frog. The use of preconstruction, during construction and post construction monitoring to measure frog distribution, abundance and habitat quality with defined thresholds will be used to measure the overall performance of the mitigation” (Lewis 2014).

The following report presents results of year 4 (2021/22) operational phase sampling, which was a recommendation of the year 3 monitoring report (see Sandpiper Ecological 2021).

## 1.1 Background

The giant barred frog is listed as ‘Endangered’ under both the NSW *Biodiversity Conservation Act 2016* (BC Act) and Federal *Environment Protection and Biodiversity Conservation Act 1999* (EPBC Act). The impact of the upgrade on giant barred frog was assessed in the Project Environmental Assessment (Sinclair Knight Merz [SKM] 2010). Following identification of potential giant barred frog habitat during the Project environmental assessment, Lewis Ecological conducted targeted surveys (in November 2011 and January/February 2013) (Lewis 2014). A population of giant barred frog was subsequently confirmed at Upper Warrell Creek and a management strategy prepared (see Lewis 2014).

Measures proposed to manage impacts on giant barred frogs included: population monitoring, pre-clearing surveys, temporary frog fencing during construction, clearing supervision, dewatering procedures (tadpole surveys) and permanent frog exclusion fence. Population monitoring was recommended to occur within a 1km transect, extending either side of the upgrade alignment, in spring, summer and autumn of Year 1 and 3 of the construction phase and years 1, 3 and 5 of the operational phase using the methods applied during pre-construction baseline surveys.

Pre-construction baseline surveys for giant barred frog were conducted between 20 September 2013 and 2 April 2014. The baseline surveys recorded 47 individuals, including 22 adults (11 females & 11 males), 8

sub-adults, and 8 juveniles. Based on these results the population of giant barred frog at the Upper Warrell Creek site was calculated as 45 adults (with a 1:1 sex ratio), 19 sub-adults, and 16 juveniles (Lewis 2014b). Geolink (2018) recalculated population size for baseline (using the same data and methods as Lewis 2014b), year 1 and year 3 construction phase samples and obtained population estimates of 41 (2013/14), 7 (2015/16), and 8 (2017/18) respectively. The results suggest a substantial decline in population between the baseline (2013/14) and year one of construction (2015/16).

Operational phase surveys recorded a population estimate of 7 individuals (95% CI of 4.8) in year 1 and 19 individuals (95% CI of 21.5) in year 3 (Sandpiper Ecological 2019, 2021). The recorded population increase in year 3 was attributed to favorable breeding conditions between February 2020 and April 2021 (Sandpiper Ecological 2021). To track population trends more closely Sandpiper Ecological (2021) recommended that additional surveys be undertaken in year 4 (i.e. 2021/22). These surveys were to apply the same methods and effort as previous operational samples focusing only on the Upper Warrell Creek site.

During early construction work *Mixophyes* spp. tadpoles were recorded at Butchers Creek (Geolink 2015). There was some conjecture about the identification of tadpoles and targeted surveys for adult frogs and further consultation with frog specialists was undertaken in an attempt to confirm the identification. The final consensus was that the tadpoles were great barred frog (*Mixophyes fasciolatus*) and the giant barred frog was unlikely to occur at Butchers Creek (see Geolink 2015; Lewis 2015). Nonetheless, a precautionary approach was adopted and the Butchers Creek site was included in population monitoring (Geolink 2016). No giant barred frogs were recorded at Butchers Creek during the construction phase, or in year one of the operational phase (Geolink 2018; Sandpiper Ecological 2019).

## 1.2 Study area

The WC2NH project covers a total length of 19.75km and extends from Warrell Creek in the south to Nambucca Heads in the north (Figure 1). The alignment bypasses the town of Macksville and the northern section traverses Nambucca State Forest. The two sample sites, Butchers Creek and Upper Warrell Creek, are situated near the southern end of the alignment.



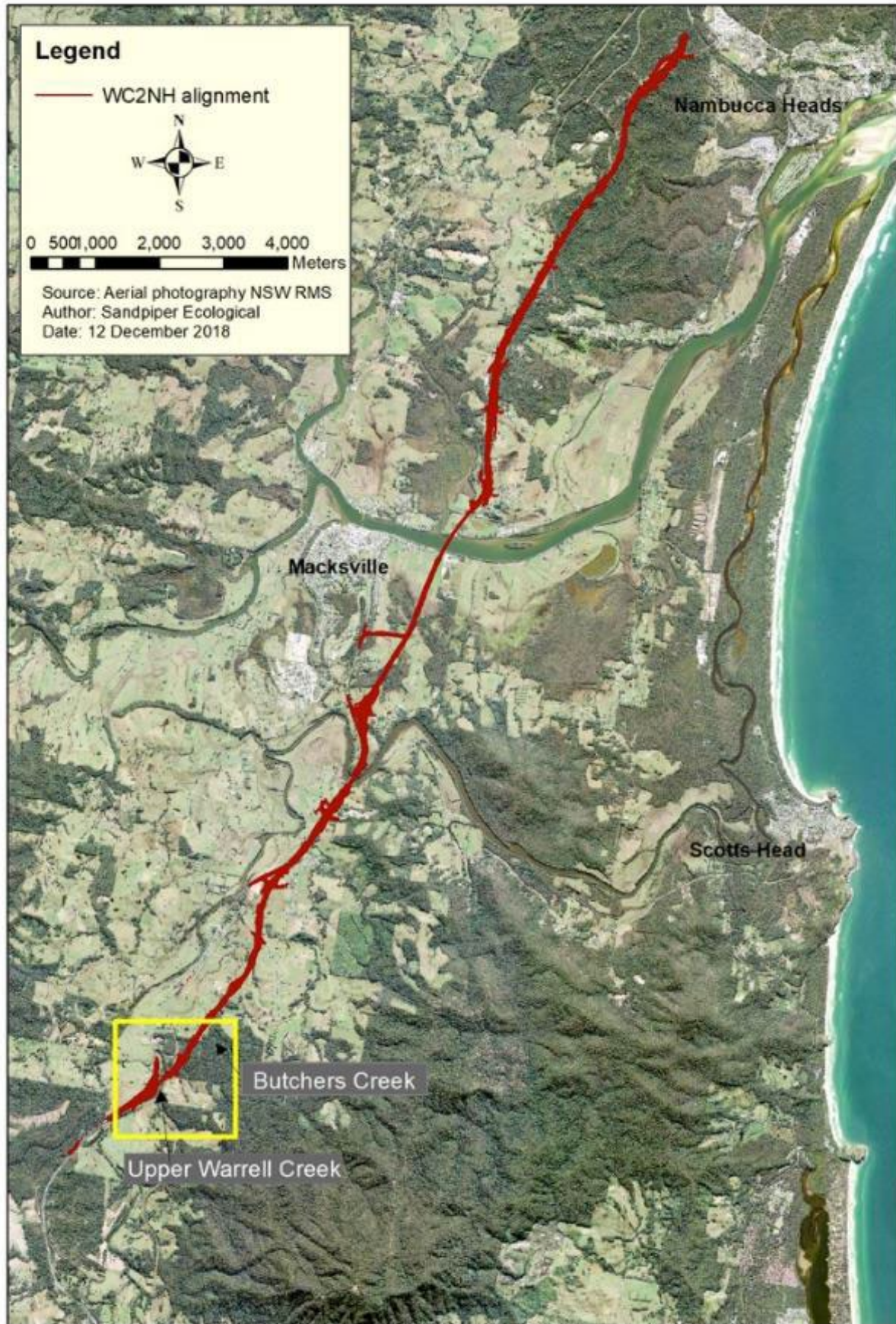


Figure 1: Location of giant barred frog sample sites in relation to the WC2NH alignment.

## 2. Methods

### 2.1 Frog survey

Frog surveys followed the method specified in the Brief and baseline population survey (Lewis 2014). The method involved:

1. Surveys were conducted on 17 and 18 November 2021 (spring survey), 9 February and 3 March 2022 (summer survey), 11 April 2022 (autumn survey, with a minimum of 16 person hours spent searching for frogs during each sample. The March 2022 survey was intended to occur in summer and was delayed due to widespread flooding on the North Coast of NSW.
2. Two-three ecologists conducted a nocturnal meandering foot-based traverse of 40 x 50m survey zones, 20 on each side of the watercourse at Upper Warrell Creek (20/side; Figure 2).
3. Each ecologist was equipped with a 200-lumen spotlight and slowly traversed the riparian zone searching for frogs and listening for calls. Giant barred frog calls were broadcast through a 5-watt megaphone for five minutes within each zone. Both ecologists listened for call responses during and immediately after call broadcast.
4. All captured giant barred frogs were scanned with a Trovan Nanotransponder to determine if that frog had been previously pit-tagged. If the captured individual had not been pit-tagged and was deemed a sub-adult or older (i.e. >40mm snout-vent length) a tag was inserted beneath the skin on the left side and the insertion hole sealed with vet bond. The insertion point was swabbed with disinfectant (Betadine) prior to the tag being inserted. During operational surveys prior to autumn 2021 only frogs with a SV length greater than 60mm were PIT tagged. In autumn 2021 the size limit was reduced to 40mm to ensure consistency with baseline and construction phase surveys.
5. The dorsal pattern of all captured frogs was photographed during each sample. Comparison of dorsal pattern is a way to distinguish individual frogs and was done to enable identification of untagged frogs captured in autumn 2021 and March 2022. Some frogs were not tagged in autumn 2022 due to insufficient tags, and in March 2022 due to equipment malfunction. The dorsal pattern of untagged frogs captured in autumn 2021 were compared to frogs captured in each of the 2021/22 sample periods, and the dorsal pattern of untagged frogs captured in summer 2022 was compared to frogs captured in autumn 2021 and autumn 2022.
6. Data collected on each captured frog included: Survey zone (20x50m); Distance from the stream edge measured to the nearest 0.1m; Position within the microhabitat (i.e. under litter, above litter, exposed, on rock/log); Sex (male, female, unknown); Age class (adult=>60mm; sub-adult=40-60mm; juvenile=<40mm); Snout-vent length (mm); Weight (grams); Breeding condition:
  - i. males assessed on the colouration of their nuptial pads (i.e. no colour, light, moderate, dark) in accordance with the classification developed by Lewis (2014b);
  - ii. females assessed on whether they are gravid (i.e. egg bearing, with the typically adult weighing > 100 grams) or not gravid.
  - iii. frogs with a snout vent length of <60 mm were classified as immature.

### 2.2 Chytrid sampling

Each captured giant barred frog (23 individuals) and two striped marsh frogs (*Limnodynastes peronii*) were swabbed for chytrid fungus. The swabbing method was consistent with Figure 3 and upon completion of the swab samples were placed in a cooler bag and transferred to a freezer as soon as possible. Swabs were analysed by Alex Callen from the Conservation Biology Research Group at the University of Newcastle.

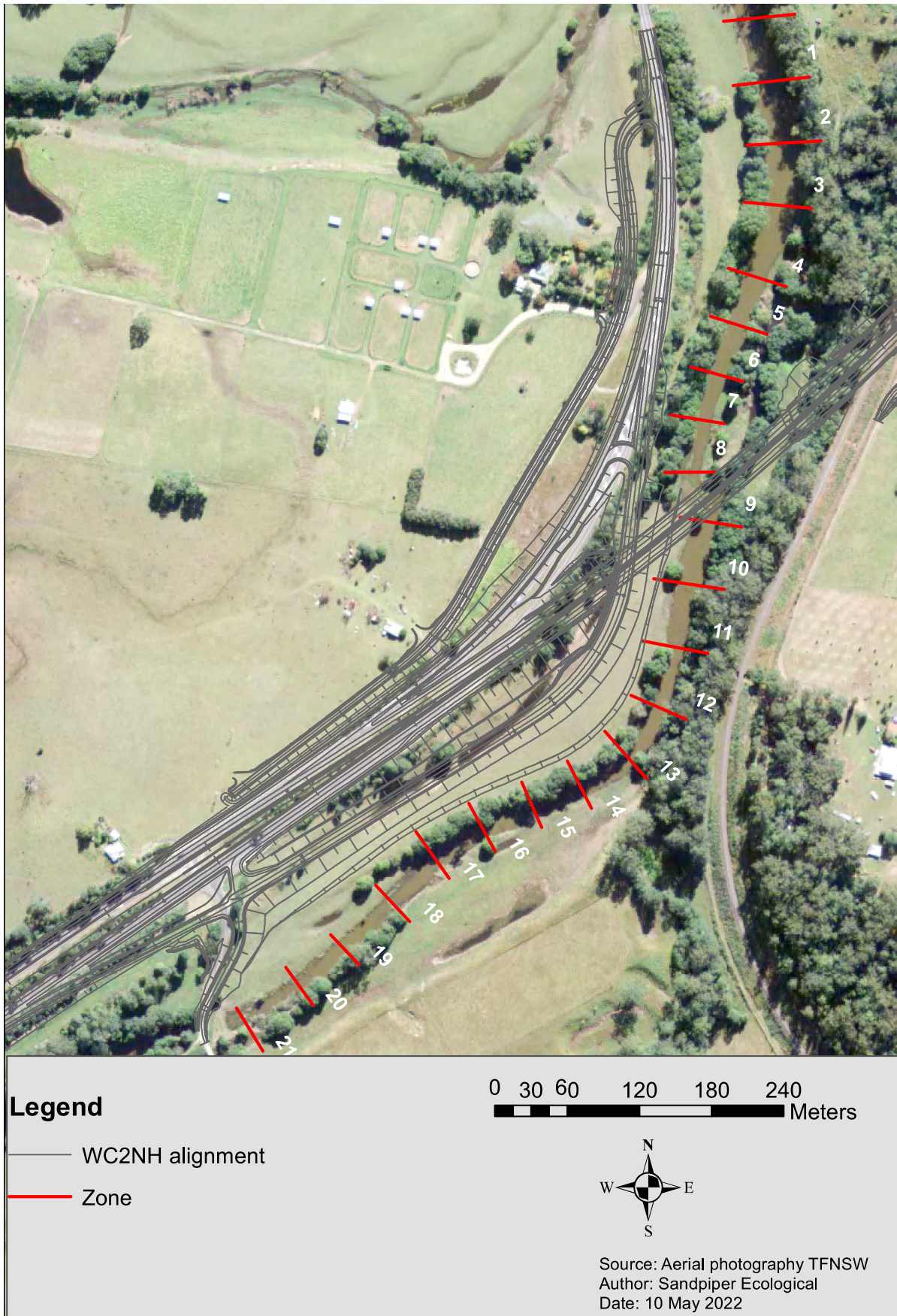
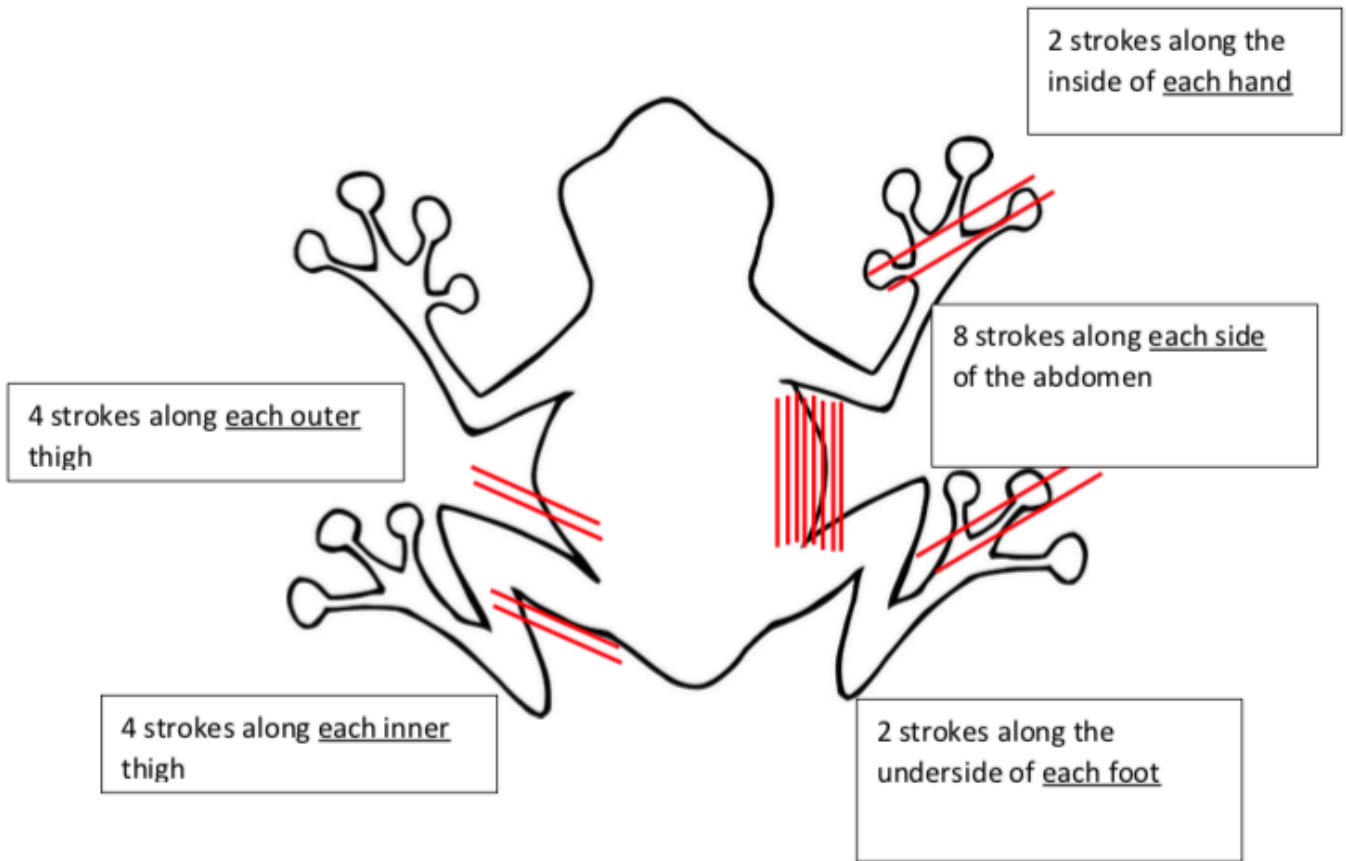


Figure 2: Survey zones within the Upper Warrell Creek and Butchers Creek sample sites.



**Figure 3:** Chytrid swabbing protocol.

## 2.6 Population estimate

The modified Petersen-Lincoln index method (that is the Petersen-Lincoln method with the Chapman estimator) was used to calculate a population estimate for year four operational phase. The method follows that applied during previous surveys (Lewis 2014; Geolink 2018; Sandpiper Ecological 2019, 2021). Juveniles, sub-adult, and non-captured individuals (i.e. calling males) were not included in the equation which is consistent with the baseline and construction phase surveys. Population estimates were calculated for all survey combinations, including spring/summer, spring/autumn and summer/autumn. The baseline population estimate was based on summer and autumn data. The equation and input data, included:

$$\hat{N} = \frac{(M + 1)(C + 1)}{(m + 1)} - 1$$

$N$  = population size

$M$  = total captured in sample 1

$C$  = total captured in sample 2

$m$  = number recaptured in sample 2

To account for uncertainty around the population estimate the confidence interval of the standard error was determined. The confidence interval is the range of values that we expect the population estimate to fall between if the survey was conducted again. For this assessment the confidence level was set at 95%. The 95% confidence interval was calculated using the following formulae:

- 95% confidence interval =  $N \pm (1.96)(SE)$

The standard error (SE) of the estimate of N was calculated using the following formulae:

- $SE = \sqrt{\frac{[(M+1)(C+1)(M-m)(C-m)]}{(m+1)^2(m+2)}}$

The population estimate derived using spring and summer data has been used in various figures as that sample included one recapture and was mostly completed before major flooding in early March 2022.

## 2.7 Data summary and analysis

Rainfall data for the year four survey and historical records were sourced from the Bellwood weather station. Individual frogs were identified by comparing PIT tag numbers recorded during this survey with those reported by Sandpiper Ecological (2019, 2021), Geolink (2018) and Lewis (2014), and dorsal photographs taken in autumn 2021 and summer 2022. The number of individuals calculated for year one construction phase might be an underestimate as it does not include individuals captured during the first autumn sample (GeoLink 2018).

## 2.8 Temporal comparison

Data collected during year four operational phase were compared to previous operational surveys, the construction phase and baseline surveys to provide a temporal comparison of frog abundance. The number of giant barred frogs detected (i.e. captured and heard calling but not captured), and captured in each time period is presented using histograms. Population estimates derived during each survey are also compared.

## 3. Results

### 3.1 Survey timing, weather conditions and effort

Weather conditions were suitable for giant barred frog surveys during all sample events (Table 1). Above average rainfall was recorded over the sample period (i.e. November 2021 to April 2022), with 574 mm falling in the 30 days prior to 3 March and 641 mm in the 30 days prior to the survey on 11 April. Several flood events occurred during the sample period, with major events prior to surveys on 9 February, 3 March and 11 April. Air temperature ranged between 17.5°C and 20°C in November, 21.8 and 23.8°C in February/March and 22.4°C in April. Wind was either absent or light (i.e. rustled leaves; Table 1). Rain or showers occurred during the spring survey only. Survey effort at Upper Warrell Creek ranged from 15.5 person hours in summer (Feb & Mar combined) to 18 person hours in autumn (Table 1).

**Table 1:** Weather conditions and survey effort recorded during the year 4 2021/22 giant barred frog survey at Upper Warrell Creek. Rainfall data were sourced from the Bellwood weather station. PH = person hours; Wind categories = 0 - no wind, 1 - rustles leaves, 2 - branches moving, 3 - canopy moving; RH = relative humidity; Rainfall = mm; Temp = °C; Dew Point = °C

Season	Date	Start/Finish	Observers	PH	Rainfall	Rainfall (prev 24hr)	Rainfall (prev 7 days)	Rainfall (prev 30 days)	RH	Temp	Dew point	Wind
Spring	17/11/21	2000-2400	DR/LA	8	Showers	Nil	0	49	NR	17.5	14.8	0
	18/11/21	2000-0015	DR/LA	8.5	Nil		0	49	NR	20	16	1
Summer	9/2/22	2000-2345	DR/LA	7.5	Nil	1	90	184	NR	23.8	18	0
	3/3/22	1945-2345	LA/AE	8	Nil	4	349	574	NR	21.8	18.9	0
Autumn	11/4/22	1745-2345	DR/LA/AE	18	Nil	22	46	641	75	22.4	18.2	0

### 3.2 Frog surveys

#### 3.2.1 Abundance

A total of 25 giant barred frogs were recorded at Upper Warrell Creek during the year four operational phase surveys (Tables 2 & 3). Captures included 17 adults (Snout-vent length >60mm), six sub-adults (S-V length 40-60mm), and two juveniles (S-V length <40mm). Two individuals, both calling males, were not captured. Both were recorded calling from concealed positions on the opposite creek bank to that being sampled.

The age of frogs was biased towards adult frogs with 17 of the 25 individuals falling in the adult class (i.e. S-V >60mm). All sub-adult frogs had a SV length between 50 and 60 mm. (Table 2). The number, sex and age-class of individuals recorded during each survey included:

- 8 (4M & 4F all adults) in spring 2021;
- 11 (3 adult male, 4 adult female, 2 juvenile, 2 sub-adult) in summer 2022; and
- 12 (1 adult male, 1 adult female, 4 sub-adult) in autumn 2022.

Confirming the sex of non-calling adult frogs is difficult and, in the absence of calls, the sex of adult frogs was based on snout-vent length and weight. Using these criteria, nine adult female frogs were recorded. Seventeen frogs were PIT tagged, eight in spring, three in summer, and six in autumn. An additional four individuals (2 adults, 1 sub-adult & 1 juvenile), captured on 3 March 2022 had their dorsal pattern photographed due to equipment malfunction (Plates 1 & 2).

**Table 2:** Data recorded for giant barred frogs captured or heard calling during the year 4 (spring 2021 to autumn 2022) operational phase monitoring survey at Upper Warrell Creek. + = positive chytrid detection; - = possible chytrid detection; NC = not captured; NA = not applicable.

Season	Date	Frog # & Chytrid	Sex**	Age***	S/V length	Weight	Breeding condition#	New tag or recapture	Microchip ID (new or re-capture)
Spr	17/11/21	1 +	Female	Adult	98.1	122	Gravid	New tag	956000010433901
Spr	11/7/21	2	Female	Adult	87.3	88		New tag	00077E8fef
Spr	18/11/21	3 +	Male	Adult	66.8	36	Moderate	New tag/recapture	11419351 (nil)
Spr	18/11/21	4 -	Male	Adult	63.5	42	Dark	New tag/recapture	11425829
Spr	18/11/21	5 +	Male	Adult	65.8	38	Dark	New tag	11423017
Spr	18/11/21	6 -	Male	Adult	73.8	48	Dark	New tag	11408672
Spr	18/11/21	7 -	Female	Adult	76.1	50	Moderate	New tag	11459761
Spr	18/11/21	8 +	Female	Adult	92.5	122	Gravid	New tag	11432455
Sum	9/2/22	9	Ukn	Juvenile	38.5	17	N/A	NA	NA
Sum	9/2/22	10	Female	Adult	86.4	95	Gravid	Recapture	11459761
Sum	9/2/22	11	Ukn	Sub adult	53.9	18	N/A	New tag	11425922
Sum	9/2/22	12	Male	Adult	76	58.3	Dark	New tag	11427483
Sum	9/2/22	13	Male	Adult	N/A	N/A		N/A	N/A
Sum	9/2/22	14	Female	Adult	79.5	80		New tag	11431052
Sum	3/3/22	15	Male	Adult	N/A	N/A		N/A	N/A
Sum	3/3/22	16 -	Ukn	Sub adult	50.3	23.5	N/A	no tag	N/A
Sum	3/3/22	17	Female	Adult	119	96.3		no tag	N/A
Sum	3/3/22	18	Ukn	Juvenile	36.6	19	NA	N/A	N/A
Sum	3/3/22	19	Female	Adult	104	90.6	Gravid	no tag	N/A
Aut	11/4/22	20	Ukn	Sub adult	52.9	22	N/A	New tag	11423778
Aut	11/4/22	21	Female	Adult	91.4	130	Gravid	New tag	11432288
Aut	11/4/22	22	Ukn	Sub adult	53.1	23	N/A	New tag	11450114
Aut	11/4/22	23 -	Ukn	Sub adult	55.2	25	N/A	New tag	11427302
Aut	11/4/22	24 -	Male	Adult	68.5	42	Moderate	New tag	11433481
Aut	11/4/22	25 +	Ukn	Sub adult	59.7	32	N/A	New tag	11421640

**Table 3:** Data recorded for Frog # 10-21 captured or heard calling during the autumn 2021 survey at Upper Warrell Creek. HC – heard calling; NC – not captured; NR = not recorded

Frog ID	Easting	Northing	Zone	Creek side	Distance to edge (nearest 0.1m)	Position in micro-habitat*	Comments
Frog 1	489317	6594399	6	Middle island (south)	4.0	On leaf litter beneath sticks	
Frog 2	489315	6594411	6	Middle island (South bank)	0.3	Beneath <i>Persicaria</i> spp.	
Frog 3	489264	6594375	7	South bank	9.0	Leaf litter	Recapture - Frog #20 originally caught in autumn 21; identified from dorsal pattern
Frog 4	489302	6594463	5	South bank	3.0	Leaf litter	Recapture - Frog #21 originally caught in autumn 21; identified from dorsal pattern
Frog 5	489303	6594464	5	South bank	6.0	Leaf litter	
Frog 6	489318	6594476	4	Southern	0.8	Leaf litter	
Frog 7	489316	6594480	4	South	0.1	Waters edge	
Frog 8	489265	6594355	7	South	7.0	Leaf litter	
Frog 9	489304	6594471	4	South bank	6.4	Leaf litter	
Frog 10	489320	6594483	4	South bank	0.5	Bare ground on bank	
Frog 11	489312	6594467	4	South bank	0.9	Leaf litter, base of tree	
Frog 12	489320	6594508	4	South bank	2.3	Leaf litter	
Frog 13	498347	6594463	4	Middle island	Calling	N/A	Not captured heard calling
Frog 14	489261	6594334	8	South bank	8.0	Leaf litter	
Frog 15	489326	6594489	4	South bank past island northern point	Calling	N/A	Calling, waypoint estimated
Frog 16	489302	6594240	10	northern bank	8.5	Leaf litter beneath fallen branches	photo taken, copper blotches present
Frog 17	489281	6594173	11	northern bank	3.2	Leaf litter covered in mud from flood	photo taken, copper blotches present
Frog 18	489269	6594152	11	northern bank	4.5	Bare ground in flood area beneath tree	photo taken, copper blotches present
Frog 19	489259	6594087	12	northern bank	0.6	Bare ground beneath log	photo taken, copper blotches present
Frog 20	489261	6594348	7	South bank	8.5	Leaf litter	Copper blotches, photo DR
Frog 21	489293	6594459	5	South bank	3.6	Scattered leaf litter	Copper blotches, photo DR
Frog 22	489266	6594367	7	South bank	6.0	Leaf litter beneath foliage	Copper blotches, photo DR
Frog 23	489265	6594124	12	North bank	4.5	Bare dirt beneath log	Copper blotches, photo DR
Frog 24	489257	6594076	13	North bank	9.0	Bare dirt, sparse litter	Lots of copper blotches, photo DR
Frog 25	489279	6594147	11	North bank	7.0	Scattered leaf litter beneath debris	Copper blotches, photo DR

\*Microhabitat: under leaf litter, under veg, on leaf litter, exposed, on a log/rock etc.





**Plate 1:** Dorsal photographs of frog #16 (left) and 17 (right) taken during the summer 2022 giant barred frog survey at Upper Warrell Creek.



**Plate 2:** Dorsal photographs of frog #18 (left) and 19 (right) taken during the summer 2022 giant barred frog survey at Upper Warrell Creek.

### 3.2.2 Recaptures

Three recaptures were recorded, two in spring, and one in summer. The two recaptures in spring were individuals initially captured in autumn 2021, and identified from dorsal pattern. The recapture in summer 2022 was initially captured (and tagged) in spring 2021. The spring recaptures were likely male frogs and the summer recapture was a female. Spring recaptured frogs had increased in S/V length by 3.7 and 3.8 mm respectively and in weight by 10 and 11.5gr respectively (Table 4). The female frog recaptured in summer had increased in S/V length by 10.3 mm (13%), and weight by 45gr (90%). This individual was recaptured less than 5m from the original capture location. The two frogs initially captured in autumn 2021 and recaptured in spring 2021 were both recaptured on the same bank and had both moved 120m and 10m upstream.

**Table 4:** Recaptured frogs recorded in year 4 at Upper Warrell Creek. S/V = snout/vent length (mm), Wgt = weight (gr), Breed Cond = breeding condition, Mod = moderate.

Frog No.	Frog ID	Initial capture data						Recapture data					
		Date	Easting	Northing	S/V	Wgt	Breed	Date	Easting	Northing	S/V	Wgt	Breed
3	#20/11 419351	15/4/21	489307	6594481	63.1	26	NA	18/11/21	489264	6594375	66.8	36	Mod
4	#21/11 425829	15/4/21	489302	6594475	59.7	30.5	NA	18/11/21	489302	6594463	63.5	42	Dark
7 & 10	114597 61	18/11/21	489316	6594480	76.1	50	NA	9/2/22	489320	6594483	86.4	95	Gravid

### 3.2.3 Capture location

All frogs were captured within riparian forest on the primary bank. The capture distance from water ranged from 0.1m to 9m with a mean of 4.51m. There was a notable difference in the mean capture distance from water for the three age classes. Mean values were 3.83m for adults, 5.9m for sub-adults and 5.45m for juveniles. All individuals were captured on bare earth, scattered leaf litter or leaf litter (Table 3).

### 3.2.4 Distribution

In year four, giant barred frogs were recorded in nine of the 21 survey zones, with individuals distributed from zone 4 to zone 13 a distance of approximately 470m (Figure 3). The highest number of frogs was recorded in zone 4 (9 frogs), followed by zone 7 with four frogs. Two individuals were recorded in zones 6, 11, 12 and 13. Eighteen of the 25 captures were recorded downstream of the alignment. Frogs were recorded on both the north and south banks. Upstream of the alignment all individuals were on the north bank, whilst downstream most were on the south bank.

Three recaptures (frogs 1, 2 & 3) were recorded during the survey, all in spring 2021. Frog number 3, an adult male, was recaptured in zone 20, 880m upstream from its original capture point in zone 3. Frogs two and three were initially tagged during the construction phase and have been captured on four occasions. Both individuals have always been captured in zone 5 or on the boundary of zones 4 and 5.

### 3.2.5 Population estimate

The adult giant barred frog population estimate for Upper Warrell Creek in year four operational phase using the spring and summer samples was estimated at 21.5 with a 95% confidence interval of 17.38

(Table 4). This suggests there is a 95% chance that the adult population within the 1km transect at Upper Warrell Creek is between 4.12 and 38.88.

The population estimate using the summer and autumn data was 29 with a 95% confidence interval of 26.28, and the population estimate using spring and autumn data was 26 with a 95% confidence interval of 28.79 (Table 5).

**Table 5:** Population estimate of adult giant barred frogs and 95% confidence interval after the conclusion of year four operational phase giant barred frog monitoring at Upper Warrell Creek.

Comparison	Population estimate	95% confidence interval
Spring and summer	21.5	17.38
Summer and autumn	29	26.28
Spring and autumn	26	28.79

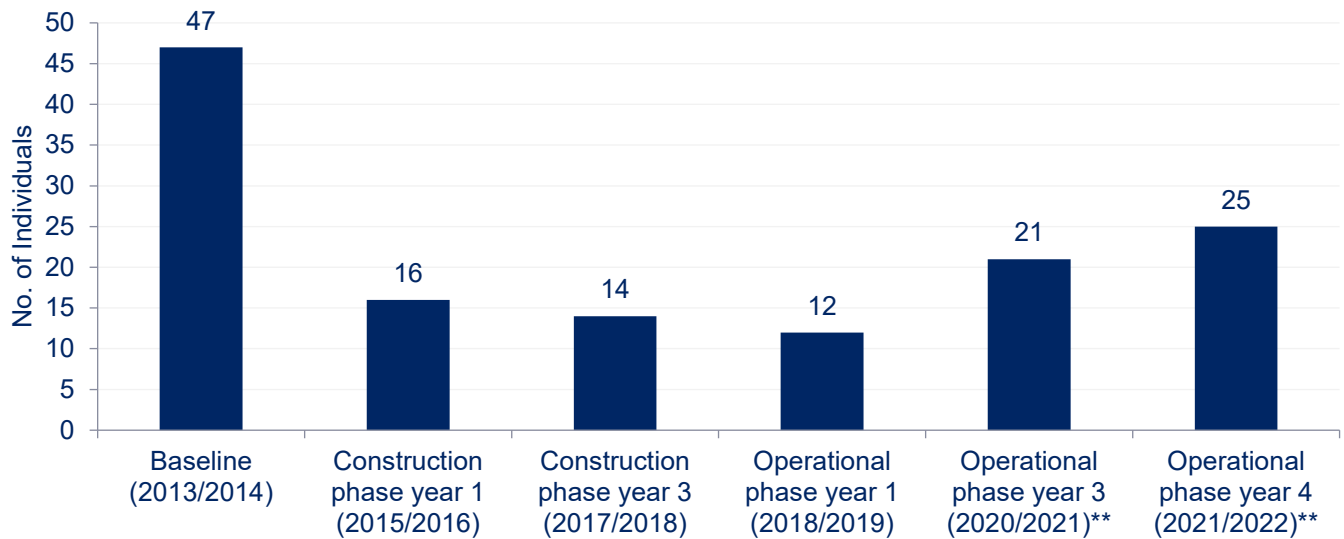


Figure 4: Location of frogs captured during the year 4 giant barred frog survey at Upper Warrell Creek.

## 3.5 Temporal comparison

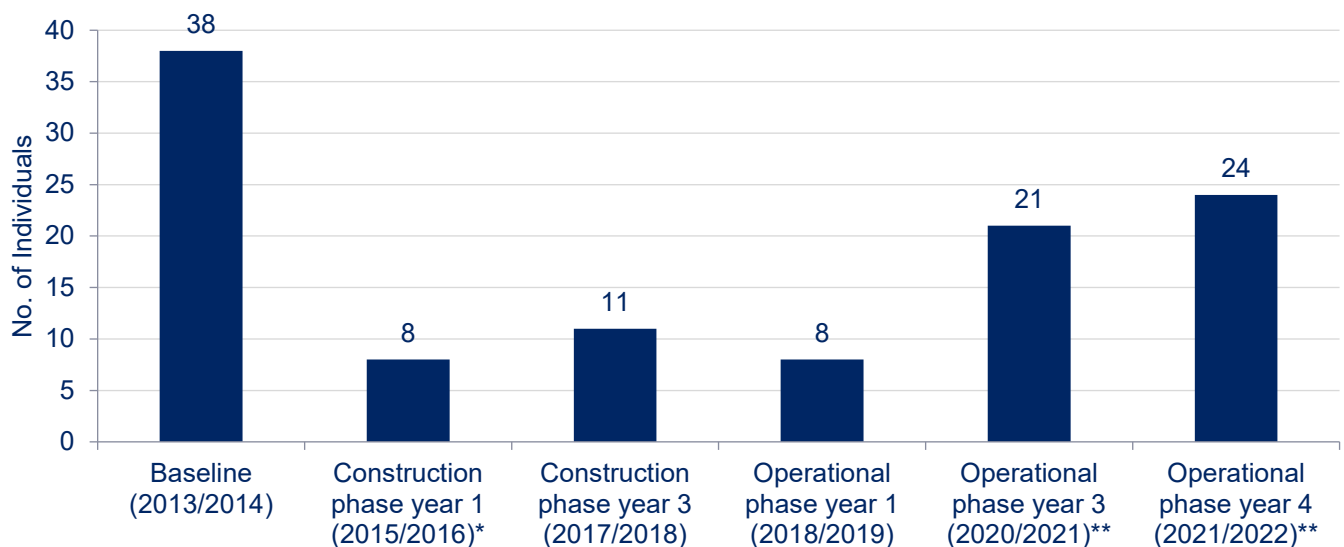
### 3.5.1 Frog surveys

The total number of giant barred frogs captured during each sample period declined substantially between baseline and year one of the construction phase. A more gradual decline was evident from year one construction phase, where 16 detections occurred, to year one operational phase, where 12 detections occurred. Captures during the operational phase have increased from 12 in year one to 21 in year 3 and 25 in year 4 (Figure 4).



**Figure 5:** Total number of giant barred frog recorded in each of five sample periods at Upper Warrell Creek. Values include multiple recaptures of the same individual and calling males that were not captured. \*\* could include recapture of unmarked sub-adults.

The number of individual frogs captured between baseline and year one construction phase surveys declined from 38 to eight and remained stable over the construction and year one operational phase surveys. The number of individual frogs increased to 21 during the year three operational phase survey and to 24 in year 4 operational phase (Figure 5).



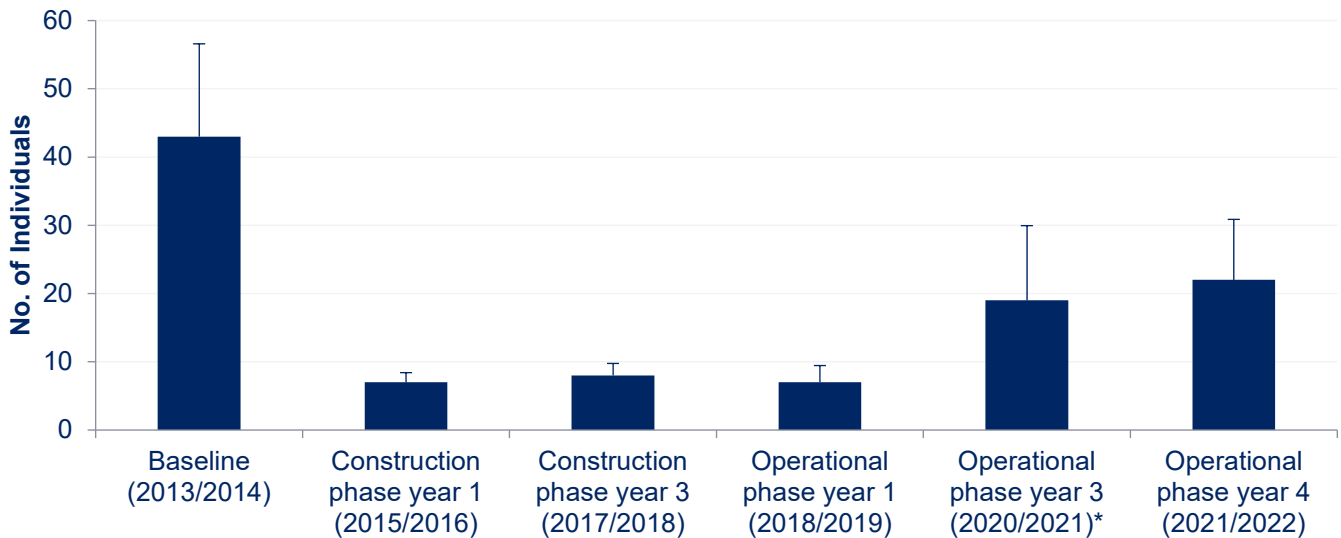
**Figure 6:** Number of individual giant barred frogs recorded over five sample events at Upper Warrell Creek. \*Year one construction phase number may be an underestimate as it does not include frogs recorded in autumn 2015 (GeoLink 2018); \*\* could include recapture of unmarked sub-adults from summer survey.

### 3.5.2 Population estimate

Comparison of adult population estimates across the five sample periods shows a decline at the Upper Warrell Creek site from baseline through the construction phase and into year one of the operational phase (Table 6, Figure 6). The population estimate of 43 adult frogs in 2013/14 declined to seven in year one of the construction phase with estimates of eight and seven recorded in year 3 construction phase and year one operation phase respectively (Table 6, Figure 6). The population increased in years three and four of the operational phase with population estimates of 19 and 21 adult frogs respectively.

**Table 6:** Population estimates of adult giant barred frog at Upper Warrell Creek prior to construction (Lewis 2014), during construction (GeoLink 2018) and operational phase (Sandpiper 2019). GBF = giant barred frog.

Parameter	Baseline (2013/2014)	Year 1 CP (2015/2016)	Year 3 CP (2017/2018)	Year 1 OP (2018/2019)	Year 3 OP (2020/2021)	Year 4 OP (2021/2022)
GBF population estimate	43	7	8	7	19	21.5
95% confidence interval	26.6	9.77	10.46	4.8	21.46	17.38



**Figure 7:** Adult population estimates (+ standard error) at Upper Warrell Creek during baseline (Lewis 2014), construction phase (GeoLink 2018), year one operational phase (Sandpiper Ecological 2019), year three operational phase monitoring (Sandpiper Ecological 2021) and year four operational phase (this study). Note: Operational phase year 3 population estimate is based on spring/autumn data, operational phase year 4 population estimate is based on spring/summer data, all other estimates based on summer/autumn data.

### 3.6 Chytrid sampling

Analysis of swabs identified five confirmed positive samples and six possible positive samples (Table A1, Appendix A). All samples were contaminated with dirt and organic material, which hampered the analysis (A. Cullen pers comm). Contamination presumably occurred from soil and organic material collected whilst catching the frogs. Four of the eight frogs captured in spring (November) returned positive results, with a further three returning possible results. Three of the remaining four positive (1 sample)/possible (2 samples) results were recorded in autumn (April). Of the three recaptured frogs one (Frog #3) returned a positive result, and one (Frog #4) returned a possible result. Both these individuals were originally captured in autumn 2021 (i.e. year three survey).

## 4. Discussion

### 4.1 Giant barred frog population

Year four operational phase giant barred frog monitoring at Upper Warrell Creek has provided further evidence of a population increase initially documented in year three (Sandpiper Ecological 2021). Using all possible sample combinations, the year four population estimate ranged from 21.5 to 28.79 individuals. The lower estimate of 21.5 individuals calculated from surveys in spring and summer has been adopted as it is based on data predominantly collected before the onset of flooding in autumn and it included one recapture. Using data collected prior to flooding reduces the potential influence of flood movement on calculations.

The cohort of similarly sized immature frogs that dominated samples in summer and autumn 2021 had most likely moved into the adult size class in 2021/22, with most adult frogs in the 60-90mm S-V range. Maas and Passioura (1999) suggested that giant barred frogs reach maturity at the end of their first year. This is consistent with our findings at Upper Warrell Creek where most of the adult frogs recorded in spring and summer 21/22 had likely metamorphosed in spring 2020. Based on growth rates it was suggested that the age cohort recorded in 2021 may breed in the 2021/22 breeding season. Whilst this is possible minimal evidence of breeding, such as calling males, was recorded. Nonetheless, the population contained individuals from all size classes, including two juvenile frogs.

A high abundance of invertebrates, the main prey for giant barred frogs (see Lemckert & Shoulder 2008), over the previous 12 months (pers obs), is likely to have increased growth rates. The female frog captured in November 2021 and again in February 2022 provides evidence of the rapid growth of adult frogs. Over a period of 82 days this frog almost doubled in weight and increased in length by 10mm.

Uncertainty remains about whether frogs within the study area have breed in that area or emigrated from upstream. Movement of frogs into the study area by flood remains likely and it stands to reason that more frogs will wash into the study area during productive breeding years, such as 2020 and 2021. The decline in recaptures in 2021/22 may also be due to flood movement with frogs equally likely to be washed out of the study area. Juvenile frogs may be particularly susceptible to flood transportation due to their small size (Koch & Hero 2007).

Results from the 20/21 and 21/22 breeding seasons are contrary to the year one operational phase surveys when recaptures accounted for 50%, 75% and 33% of all captures in spring, summer and autumn respectively (Sandpiper Ecological 2019a), and all individuals captured in spring 2020, prior to flooding, were recaptures (Sandpiper Ecological 2020). There appears to be a correlation between declining recaptures and flood frequency. Prior to December 2020 the majority of captures occurred on the north bank of zones 4, 5 and 6, particularly in the low-lying part of zone 6. Since that time occurrence of frogs in that area has been patchy and there have been no recaptures. Not surprisingly, floods heavily impact the low-elevation north bank in zones 4, 5 and 6.

A key assumption of the population estimate procedure is limited immigration, emigration and mortality during the sample period (Fowler *et al.* 1999). Movement of frogs into and out of the sample population is a limitation of the monitoring program. However, such movement has been consistent across all samples including the baseline. Given the variability of the frog population within the study area it seems likely that repeat sampling over many years both within and upstream of the study area would be required to determine how floods and insitu recruitment influence local abundance. Determining larger scale population trends is typically beyond the scope of normal operational phase monitoring programs.

Notwithstanding the above limitation movement of frogs in and out of the study area should be expected given the obvious connection with suitable habitat upstream. The importance of movement on the abundance of frogs in the study area is secondary to determining if the area can support at least part of the local giant barred frog population in the long-term. Since construction of the highway obvious changes in

habitat have occurred. Some of which has been directly due to construction, whilst others are due to the exclusion of cattle and clearing by land owners.

## 4.2 Distribution and movement

No frogs were recorded to have moved beneath the highway in year four of the operational phase. The presence of frogs in nine of the 21 zones in 2021/22 indicates that the species continues to occur throughout the study area, albeit in fewer zones than baseline surveys. The majority of records occurred within zones 4-7, which is consistent with previous surveys (Lewis 2014, Geolink 2016, 2018; Sandpiper Ecological 2019). Contrary to years 1-3 of operational phase monitoring six individuals were captured on the north bank of zones 11-13. The sudden appearance of frogs in those zones is attributed to flood movement.

## 4.3 Chytrid analysis

Lewis (2014) swabbed 17 frogs for chytrid in summer 2014, and Geolink (2018) swabbed 10 frogs in spring/summer 2015/16, and 11 frogs in spring/summer 2017/18. Four of the 38 individuals swabbed between 2014 and 2018 tested positive for chytrid fungus, however, only five of the 38 tests were collected in spring, with two collected in autumn. All remaining samples were collected in summer. As chytrid prefers cooler temperatures (DoEE 2016) it is likely that at low elevation sites, such as Upper Warrell Creek, infection rate will be higher in late winter and early spring (A. Cullen pers comm). To date, no samples have been collected in late winter/early spring, although, the 2021 samples were collected towards the end of what was a cool spring. Importantly, the bias of pre-construction and construction phase sampling towards summer (when 82% of samples were collected) may have masked the true scale of infection. During 2021/22 sampling only one possible detection occurred from the nine frogs swabbed in summer, compared to seven of the eight frogs swabbed in spring.

The results of chytrid analysis suggest that amphibian chytrid fungus (*Batrachochytrium dendrobatidis*) could be playing a role in the declining abundance of giant barred frogs at Upper Warrell Creek. The impact of chytrid fungus on amphibian populations is complex and, whilst there have been some extinctions (Lips 2016), other species continue to persist with stable infection rates following an initial die-off (Retallick et al. 2004; Newell et al. 2013). The impact of chytrid on a frog population is likely influenced by synergistic interactions with other threats (Buck et al. 2015). In addition to chytrid frogs at Upper Warrell Creek likely experience threats from pesticides, high nutrient levels, drought, changing vegetation structure, clearing of habitat and regular handling. Collectively these factors may contribute to the noted population decline. How chytrid was introduced into the population is unknown, however, its presence during the baseline survey suggests that it was introduced to the population prior to commencement of monitoring or construction.

## 5. Conclusion and Recommendations

The year four operational phase giant barred frog survey recorded an increase in abundance on year three, with a total of 25 individuals recorded. The adult population was estimated at 21 individuals, an increase of two on the year three estimate, and the highest since baseline surveys in the 2013/14 breeding season. The year four survey achieved its goal by enabling the cohort of juvenile frogs recorded in the 2020/21 breeding season (i.e. year 3 operational phase) to be tracked more closely. Data collected in year four suggests that those frogs had matured and most likely bred in the 2021/22 breeding season. The year four survey has also confirmed the continued presence of *B. dendrobatidis* infection within the population. Analysis of previous survey data suggests that the level of infection may have been underestimated.

Based on available evidence it seems likely that the giant barred frog population at Upper Warrell Creek persists with a background level of *B. dendrobatidis* infection. Chytrid is not considered to be the sole



reason for population decline, however, it may be a contributing factor with its impact exacerbated by the range of other threats present at the site.

Recommendations are included in Table 7.

**Table 7:** Recommendations

Number	Recommendation	Transport for NSW Response
2.	Continue to focus survey effort at Upper Warrell Creek as agreed following the summer 2021 population survey.	Agree.

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## Appendix A - Chytrid results

**Table A1:** Results of chytrid analysis of 25 frogs swabbed at Upper Warrell creek in the 2021/22 breeding season.

Frog No. (sample code)	Date	Cq	Cq Mean	Cq Std. Dev	Mean Bd molecules/ul	Result Interpretation
23	11/4/22		0.00	0.000	43	Non-confident detection
		43.31	43.31	0.000		
			0.00	0.000		
24	11/4/22		0.00	0.000	16	Non-confident detection
			0.00	0.000		
		49.03	49.03	0.000		
19	3/3/22		0.00	0.000	0	Inhibited
			0.00	0.000		
			0.00	0.000		
22	11/4/22		0.00	0.000	0	Negative
			0.00	0.000		
			0.00	0.000		
21	11/4/22		0.00	0.000	0	Negative
			0.00	0.000		
			0.00	0.000		
20	11/4/22		0.00	0.000	0	Negative
			0.00	0.000		
			0.00	0.000		
13	10/2/22		0.00	0.000	0	Negative
			0.00	0.000		
			0.00	0.000		
15	10/2/22		0.00	0.000	0	Negative
			0.00	0.000		
			0.00	0.000		
25	11/4/22	37.05	37.05	0.000	52255	Positive
		36.23	36.23	0.000		
		37.80	37.80	0.000		
16	3/3/22	45.28	45.28	0.000	138	Non-confident detection
			0.00	0.000		
			0.00	0.000		
18	3/3/22		0.00	0.000	0	Negative
			0.00	0.000		
			0.00	0.000		
11	9/2/22		0.00	0.000	0	Negative
			0.00	0.000		
			0.00	0.000		
9	9/2/22		0.00	0.000	0	Negative
			0.00	0.000		
			0.00	0.000		
10	9/2/22	49.75	49.75	0.000	10	Non-confident detection
			0.00	0.000		
			0.00	0.000		

Frog No. (sample code)	Date	Cq	Cq Mean	Cq Std. Dev	Mean Bd molecules/ul	Result Interpretation
2	10/2/22		0.00	0.000	0	Negative
			0.00	0.000		
			0.00	0.000		
17	3/3/22		0.00	0.000	0	Negative
			0.00	0.000		
			0.00	0.000		
12	9/2/22		0.00	0.000	0	Negative
			0.00	0.000		
			0.00	0.000		
14	9/2/22		0.00	0.000	0	Negative
			0.00	0.000		
			0.00	0.000		
8	18/11/21	35.18	35.18	0.000	20728	Positive
			0.00	0.000		
			0.00	0.000		
6	18/11/21	42.79	42.79	0.000	109	Non-confident detection
		43.03	43.03	0.000		
			0.00	0.000		
SM2	18/11/21		0.00	0.000	0	Negative
			0.00	0.000		
			0.00	0.000		
4	18/11/21	40.40	40.40	0.000	328	Non-confident detection
		42.05	42.05	0.000		
		46.61	46.61	0.000		
SM1	17/11/21		0.00	0.000	0	Negative
			0.00	0.000		
			0.00	0.000		
1	19/11/21	42.66	42.66	0.000	801402	Positive
		29.80	29.80	0.000		
			0.00	0.000		
3	18/11/21	39.55	39.55	0.000	1068	Positive
			0.00	0.000		
			0.00	0.000		
2	17/11/21		0.00	0.000	0	Negative
			0.00	0.000		
			0.00	0.000		
7	18/11/21		0.00	0.000	0	Negative
			0.00	0.000		
			0.00	0.000		
5	18/11/21	38.76	38.76	0.000	24563	Positive
		37.91	37.91	0.000		
		38.08	38.08	0.000		