Transport for New South Wales

Warrell Creek to Nambucca Heads

Giant Barred Frog Population Monitoring – Annual Report Year Five Operational Phase (2022-2023)

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Pacific Highway upgrade: Warrell Creek to Nambucca Heads (WC2NH)

Giant Barred Frog – operational phase year five annual report (2022-2023)

Sandpiper Ecological Surveys

Draft Report

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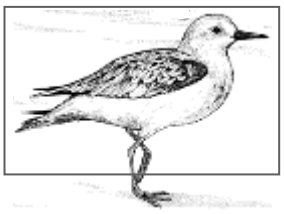

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Cover Photo: Giant barred frog (*Mixophyes iteratus*) found on leaf litter at Upper Warrell Creek.

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1 Introduction

In 2015, Transport for New South Wales (TfNSW), in conjunction with Acciona Ferrovial 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. TfNSW was responsible for managing and evaluating the effectiveness of these measures. Sandpiper Ecological Surveys (SES) was contracted by 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 giant barred frog was one threatened species identified as requiring mitigation and monitoring through the course of the project's construction and operational periods.The following annual report presents the findings of the year five operational phase giant barred frog population monitoring, and concludes the monitoring for the project as defined within the Giant Barred Frog Management Strategy (GBFMS). The objective of giant barred frog monitoring, is "to demonstrate through the life of the project that mitigation has maintained or improved population sizes and habitat of giant barred frog." (Lewis 2014b). This objective is discussed in light of findings from the baseline, construction and operational phase monitoring.

1.1 Background and monitoring framework

The giant barred frog is listed as 'Endangered' under both the NSW *Biodiversity Conservation Act 2016* (BC Act) and Commonwealth *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 2014a). A population of giant barred frog was subsequently confirmed at Upper Warrell Creek and a management strategy prepared (see Lewis 2014b).

Measures proposed to manage impacts on giant barred frogs included: population monitoring, pre-clearing surveys, temporary frog fencing during construction, clearing supervision, de-watering 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 years 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. To track population trends more closely Sandpiper Ecological (2021) recommended that additional surveys be undertaken in year 4 (i.e. 2021/22). The recommendation was approved and an annual report was produced in 2022 (Sandpiper Ecological 2022).

Pre-construction baseline surveys for giant barred frog were conducted between 20 September 2013 and 2 April 2014. The baseline surveys recorded 47 giant barred frogs, including 22 adults (11 females & 11 males), eight sub-adults, and eight juveniles. Based on these results, the population of giant barred frogs at the Upper Warrell Creek site was calculated as 45 adults (with a 1:1 sex ratio), 19 sub-adults, and 16 juveniles (Lewis Ecological 2014b). Geolink (2018) recalculated population size for baseline, 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 2013/14 and 2015/16.

During the operational phase, the population was estimated to be seven with a 95% confidence interval (CI) of 4.8 in the first year (2018/2019), increasing to 19 with a 95% CI of 21.5 in the third year (2020/2021), and 21.5 with a 95% CI of 17.38 in the fourth year (2021/2022) (Sandpiper Ecological 2019, 2021, 2022). The increased population estimate in the third and fourth operational year was attributed to favorable breeding conditions between February 2020 and April 2022 (Sandpiper Ecological 2021).

1.2 Monitoring at Butchers Creek

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). After completing the Year 3 spring operational phase survey, it was agreed with TFNSW to discontinue future monitoring at Butchers Creek. This decision was influenced by the landowner's refusal to grant entry due to heightened tree-fall risks from severe flooding. Moreover, there were no records of the giant barred frog during both construction and operational surveys. Consequently, subsequent surveys in year four and five were exclusively conducted at the Upper Warrell Creek site.

2 Methodology

2.1 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). Due to the removal of Butchers Creek as a survey site in year 3, monitoring in year 5 focused on the 1km transect at Upper Warrell Creek (Figure 2). The transect extended either side of the upgrade alignment and was divided into 21 zones as per baseline monitoring (Figure 2).

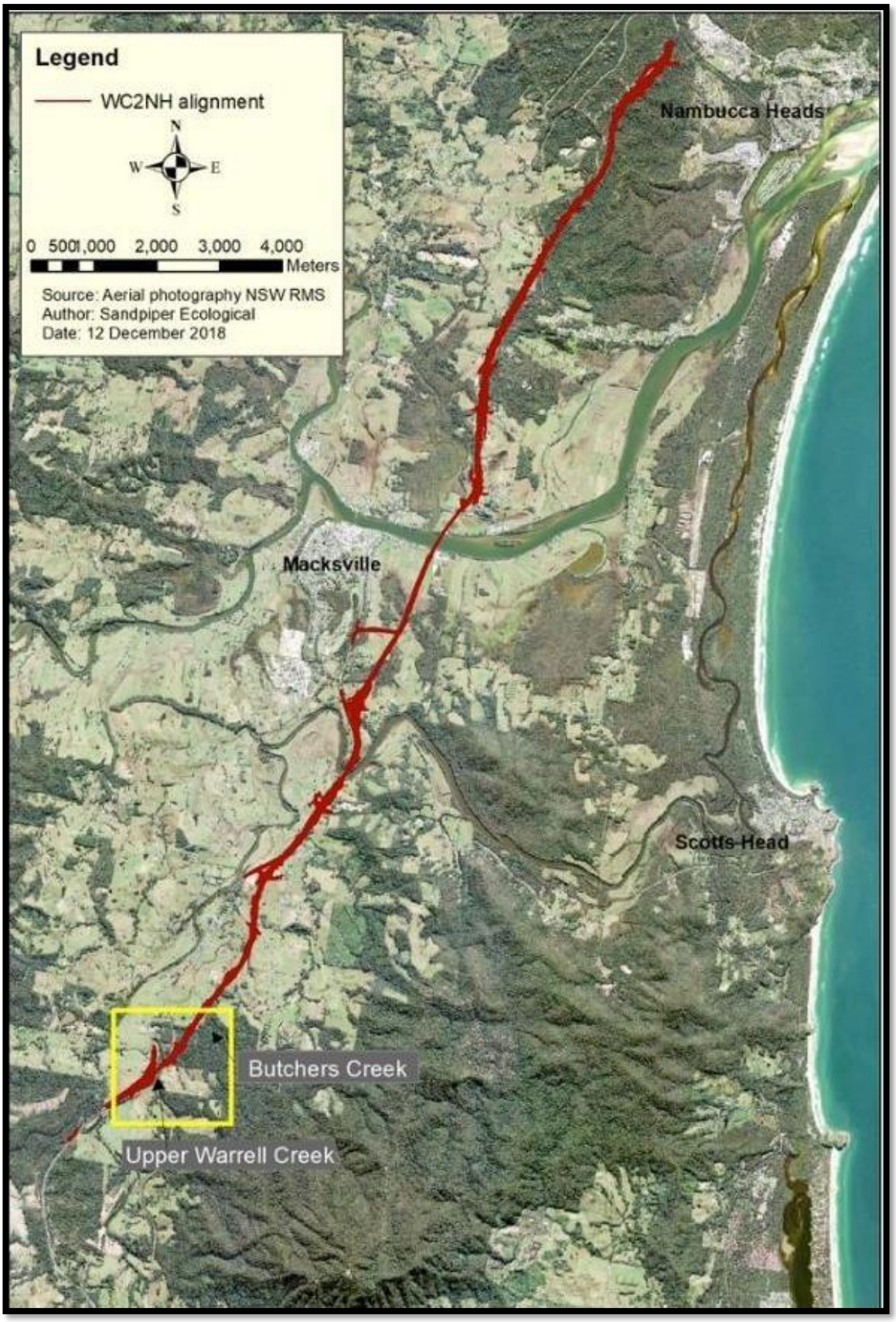

Figure 1: Location of giant barred frog sample sites in relation to the WC2NH alignment.

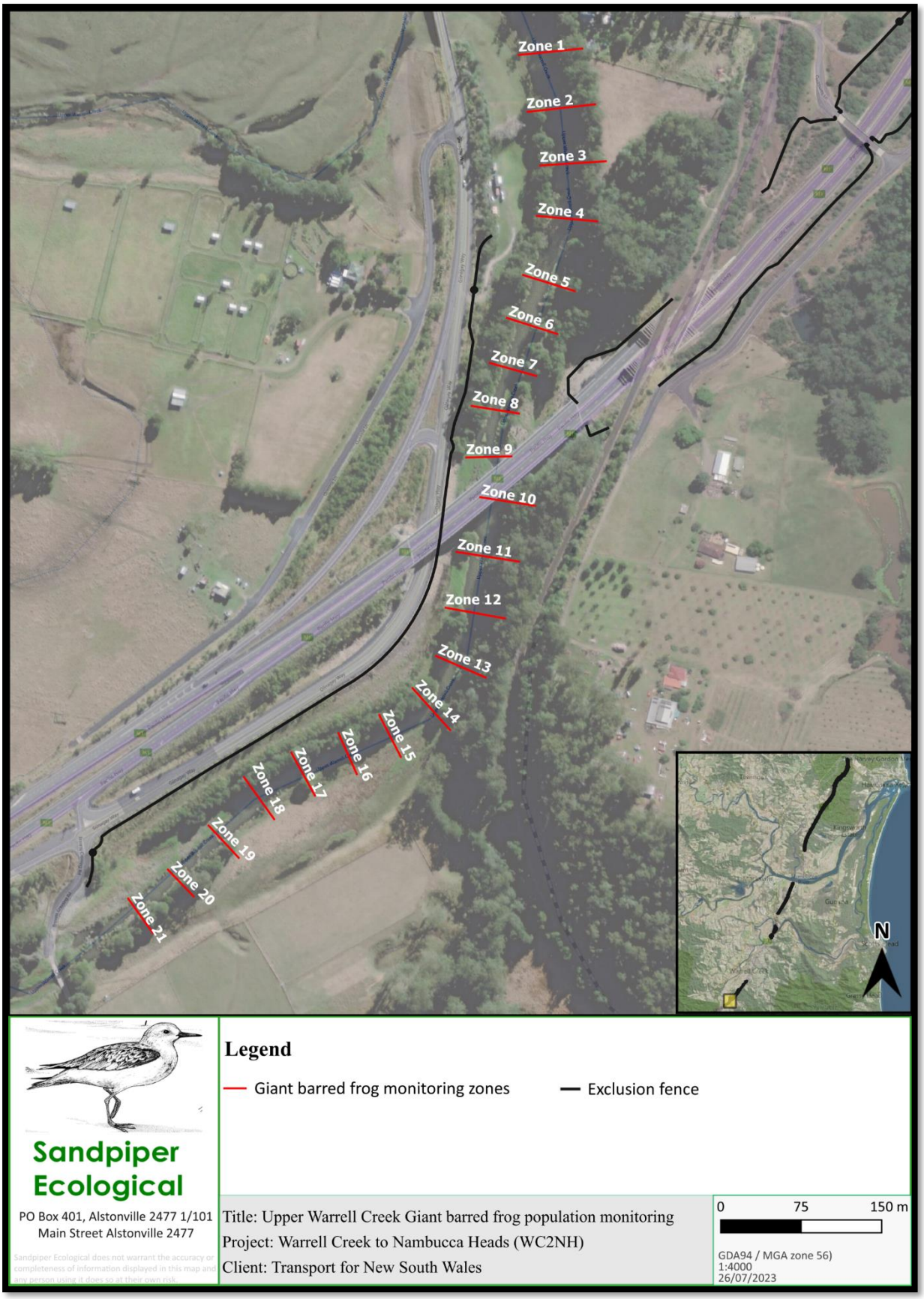

Figure 2: Survey monitoring zones at Upper Warrell Creek.

2.2 Frog surveys

Year five (2022-2023) giant barred frog surveys were undertaken across three seasons: spring 2022, summer 2023, and autumn 2023. Surveys took place within a seven-day period following rainfall events that registered more than 10 mm in a 24-hour period, as recorded by the Bellwood weather station (BOM station: 05915). Surveys followed the method specified in the Brief and baseline population survey (Lewis 2014a &b). The method involved:

1. Two to three ecologists carried out nocturnal, foot-based surveys across 21 designated sample zones. These zones spanned the riparian area extending 25 meters on either side of Upper Warrell Creek (Figure 2).
2. Each ecologist carried a 200-700 lumen spotlight and methodically traversed the riparian zone to visually identify and audibly detect frogs. A 2-watt Bluetooth speaker was used to broadcast giant barred frog calls for five minutes in each zone. Both ecologists actively listened for call responses during and immediately after the broadcast.
3. All captured giant barred frogs were scanned with a Trovan Nanotransponder to determine if that frog had been previously pit-tagged. Un-tagged frogs measuring over 40mm in snout-vent length received a subdermal pit-tag on their left side. The incision was disinfected with Betadine and sealed with vet bond. The size criterion for pit-tagging was lowered from 60mm to 40mm in Autumn 2021 to align with baseline and construction phase surveys.
4. Dorsal pattern photographs were taken of all captured frogs for individual identification, aiding in the identification of untagged records captured during Autumn 2021 and March 2022.

Data collected for each captured frog included the following variables:

- Survey zone (20x50m)
- Distance from stream edge, accurate to 0.1m
- Microhabitat location (e.g., under or above litter, exposed, on rock/log)
- Sex (male, female, unknown)
- Age class (adult >60mm, sub-adult 40-60mm, juvenile <40mm)
- Snout-vent length in mm
- Weight in grams
- Breeding condition:
    i. Males: nuptial pad coloration (none, light, moderate, dark) as per Lewis's (2014b) classification
    ii. Females: gravidity status (egg-bearing, usually weighing over 100 grams, or not)
    iii. Immature designation for frogs with snout-vent length <60mm

2.3 Tadpole survey

Tadpole surveys were undertaken during summer 2023 and autumn 2023 using the following procedure:

1. Dip-netting by two ecologists within each survey zone. Dip-netting targeting areas of undercut bank and detritus.

2. One bait trap (~300 mm x 200 mm), baited with bread, was installed within each zone for 2 -3 hours.
3. In the event of a tadpole capture the following information was recorded:
    i. Species
    ii. Survey zone (20x50m).
    iii. Sex (male, female, unknown).
    iv. Weight (grams).

Tadpoles were identified with reference to Anstis (2001, 2017).

2.4 Habitat assessment

A habitat assessment was conducted during the summer survey on 7 Feburary 2023. Habitat data recorded in each zone included:

1. Land use: Description of existing land uses e.g. grazing, dairy, horticulture, conservation, private native forestry.
2. Broad vegetation type within the immediate riparian zone (primary stream bank): riparian rainforest, dry sclerophyll, wet sclerophyll, sedgeland, grassland or cleared Land.
3. In stream physical characteristics including stream width and depth (metres), presence of pools and/or riffles, bed composition (sand, clay, rock, organic or other to be specified), and type of emergent vegetation, if present.
4. Stream bank characteristics including bank profile expressed as steep, benched or a gradual incline from the water's edge.
5. Foliage projective cover of overstorey, midstorey and ground layer vegetation on the stream bank.
6. Groundcover expressed as a percentage of vegetation, leaf litter, soil, and exposed rock.
7. Litter depth - Deep (>100 mm); Moderate (20-100 mm); Shallow (>0-20 mm); or Absent (0 mm).

2.5 Water quality sampling

Water samples and field measurements were taken at Upper Warrell Creek in zone 8 during summer and autumn of 2023 (Figure 3). Due to a change in property ownership, the sample collection site was moved approximately 100m upstream. Field physicochemical measurements, including Conductivity, pH, Temperature, dissolved oxygen and turbidity, were measured using a Horiba U-52 multiparamter probe.

Water quality parameters analysed include:

1. Heavy Metals including arsenic, cadmium, chromium, copper, lead, mercury, nickel and zinc.
2. Nutrients including Nitrogen (as N), Suspended Solids and Total Phosphorus.
3. Hydrocarbons from the following groups:
    i. Naphthalene group including TRH>C10-C16, TRH>C10-C16 less Naphthalene (F2), TRH>C16-C34, TRH>34-C40, TRH C6-C10 and TRH C6-C10 LESS BTEX (F1).
    ii. BTEX group including Benzene, Ethylbenzene, m&p-Xylenes, o-Xylene, Toluene and Xylenes – total.

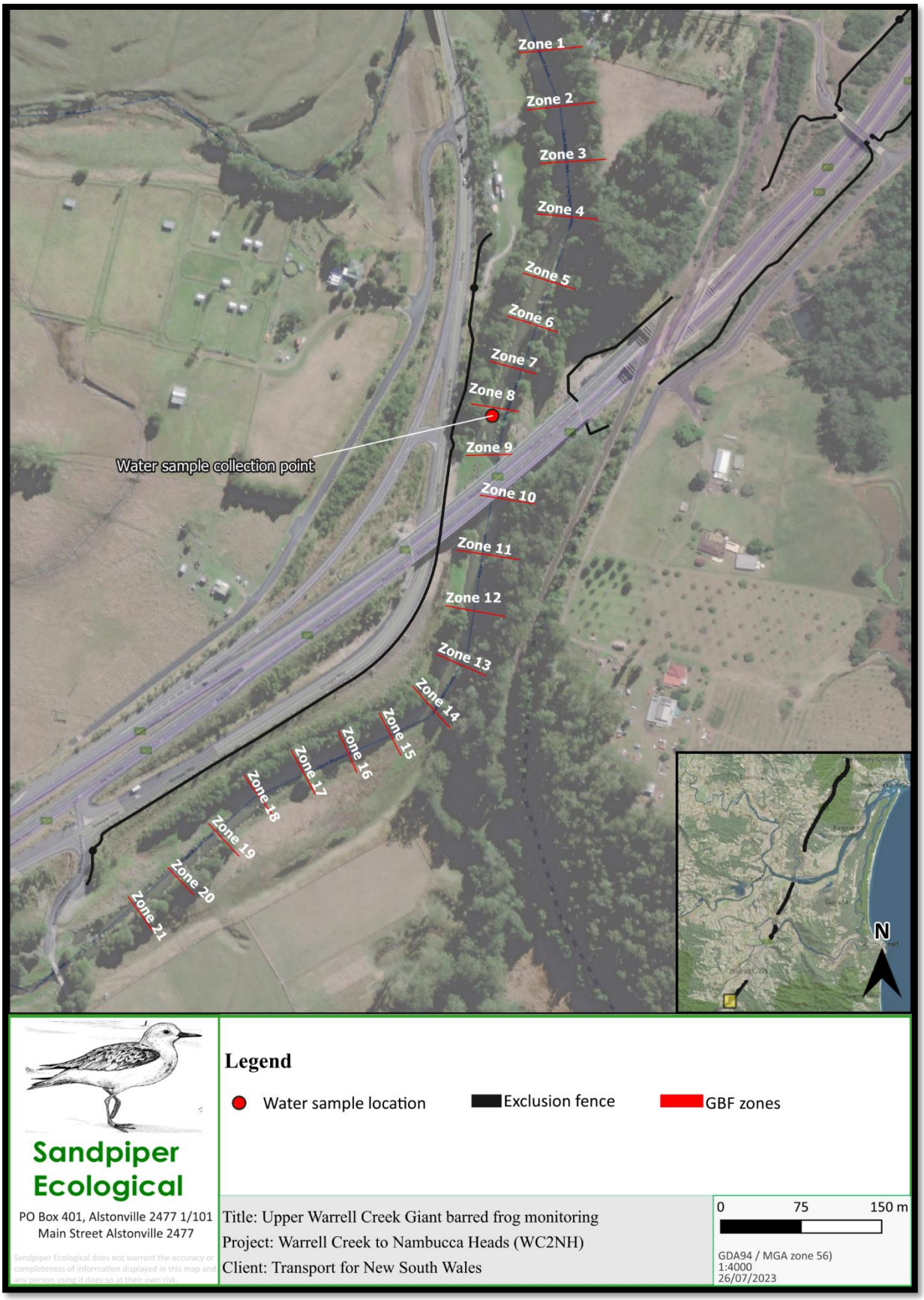

Figure 3: Location of water sampling site in relation to the survey zones at Upper Warrell Creek.

2.6 Population estimate calculation

The modified Petersen-Lincoln index method (that is the Petersen-Lincoln method with the Chapman estimator) was used to calculate a population estimate for each operational phase monitoring year. The method follows that applied during previous surveys (Lewis 2014b; Geolink 2018; Sandpiper Ecological 2019, 2021, 2022). Juveniles, sub-adult, and non-captured records (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 spring and summer 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 ± (1.96)(SE)

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

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

2.7 Chytrid sampling

Chytrid sampling was undertaken during summer of year 4 operational phase monitoring. 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 4 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.

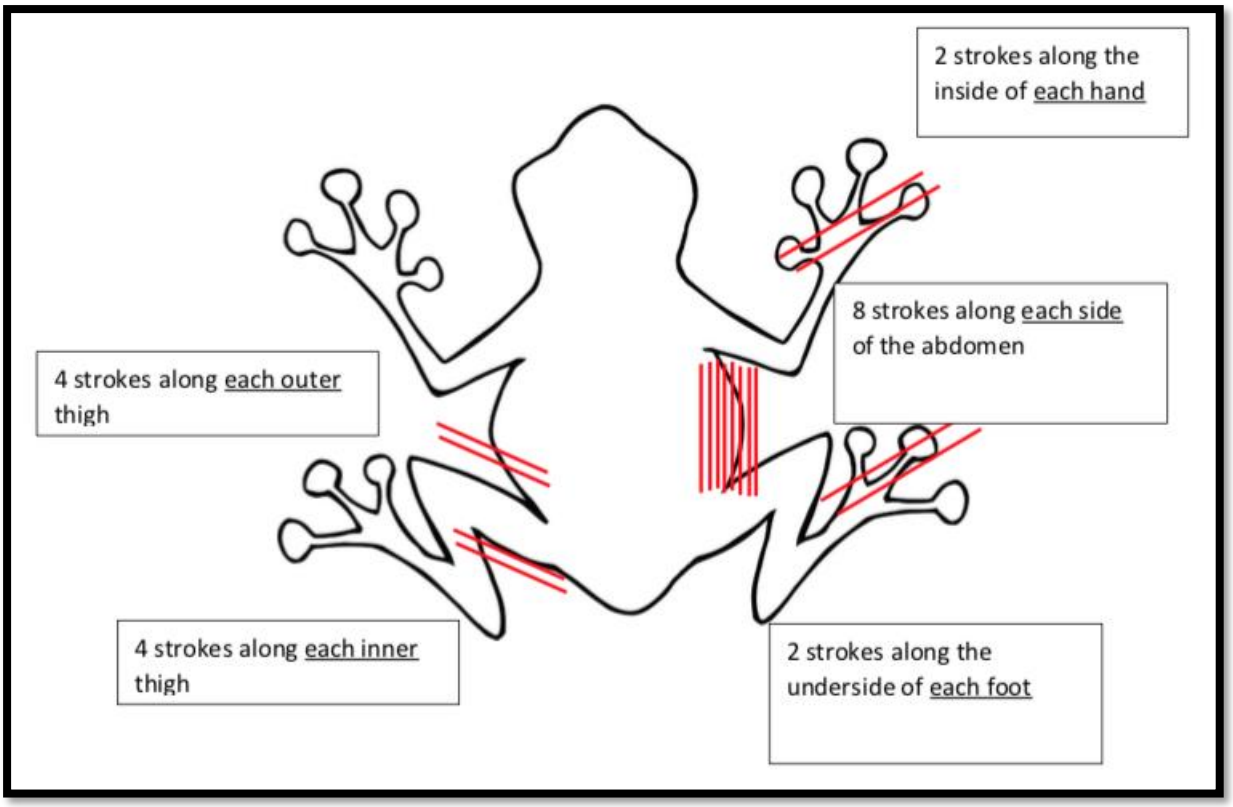

Figure 4: Chytrid swabbing protocol.

2.8 Data summary and statistical analysis

Individual frogs were identified using PIT tag numbers and comparing them with those reported by Sandpiper Ecological (2019, 2021, 2022), Geolink (2018), and Lewis (2014b). Additionally, dorsal photographs from year 4 monitoring and the surveys conducted in spring 2022 and summer 2023 were used as a secondary method of identifying potential recaptures. Confirming the sex of non-calling adult frogs is challenging. In the absence of calls, the sex of adult frogs was determined based on snout-vent length and weight.

To provide a temporal comparison of frog abundance, data collected during the year five operational phase were compared to previous operational surveys, the construction phase, and baseline surveys. The number of giant barred frogs detected (i.e., captured and heard calling) for each time period is presented using histograms. Population estimates derived during each survey are also compared. The number of records calculated for the year one construction phase might be an underestimate as it does not include records obtained during the first autumn sample (GeoLink 2018).

Rainfall data for the year five survey and historical records were sourced from the Bellwood weather station (BOM station: 05915). Rainfall events exceeding 75mm were considered to trigger potential flood events for Upper Warrell Creek following reference to the Green thighed frog management plan for WC2NH (Lewis 2013).

A linear regression analysis was performed in Excel using the Data Analysis ToolPak add-in to explore the relationship between seasonal rainfall and the number of giant barred frog records. Data on the total number of giant barred frog records, including both calling and captured individuals, were collected for each season in years 1-5 of operational montiroing. Seasonal rainfall totals for the same seasons were obtained from the

Bellwood weather station. It is imperative to note that the analysis was based on a limited dataset comprising only 12 data points, and the results should be cautiously interpreted as indicative rather than conclusive.

3 Results

3.1 Survey timing, effort and weather conditions

Rainfall varied noticeably across the surveys during the year 5 monitoring period. Although rainfall occurred before the first spring 2022 survey, it was not recorded until 9am the next day, resulting in only 7mm in the 30 days leading up to the survey. A further 19mm fell 24 hours prior to the second survey (Table 1). The summer period also experienced below-average rainfall, with only 51mm falling in the 30 days prior to the surveys (Table 1). In comparison, the first autumn survey in March 2023 was preceded by a notably higher 174mm of rainfall within the same time frame (30 days).

In terms of atmospheric conditions, relative humidity and dew point remained relatively high across the surveys, while temperatures ranged from 15.6°C to 22.4°C. Temperatures were conducive for detecting calling males on five out of seven occasions, based on the criterion of Koch & Hero (2007) that the temperature should be above 18°C. Wind conditions were also generally calm and showers did occur once during the third summer survey.

Survey effort varied slightly. The summer period recorded the highest survey effort, accumulating a total of 21.5 person-hours, followed by the autumn period with 16.75 hours and the spring 2022 period with 15.75 person-hours. Overall a total of 54 person-hours were spent conducting frog surveys at Upper Warrell Creek during year 5 population monitoring.

Table 1: Weather conditions and survey effort recorded during the year five giant barred frog surveys at Upper Warrell Creek. Rainfall data were sourced from the Bellwood weather station (BOM station: 05915). PH = person hours; Wind categories = 0 - no wind, 1 - rustles leaves, 2 - branches moving, 3 - canopy moving; RH = relative humidity (%); Rainfall = mm; Temp = ^{0}C; Dew Point = ^{0}C . Rainfall prior = rainfall recorded at the Bellwood weather station 24hours/7 days/30 days prior to the specific survey.

Season	Date	Observers	Survey	Effort (PH)	Rain (during)	Rainfall prior	Temp	RH	Dew point	Wind
Spring 22	1/12/22	LA/AE	1	7.75	Nil	0/0/7	16.8	86	14.9	2
	2/12/22	LA/AE	2	8	Nil	19/19/26	18.2	69	14.2	1
Summer 23	6/2/23	LA/AE	1	9	Nil	0/21/51	22.4	61	15.9	0
	7/3/23	LA/AE	2	8.5	Nil	0/9/51	21.9	74	16.1	0
	24/3/23	LA/AE	3	4	Showers	22/88/110	20.7	92	20.1	0
Autumn 23	29/3/23	LA/AE	1	8.5	Nil	27/105/174	19.9	73	13.8	0
	2/5/23	LA/AE/EL	2	8.25	Nil	0/14/92	15.6	54	8.2	0

3.2 Rainfall patterns

The annual rainfall in the Upper Warrell Creek area (Bellwood) has exhibited considerable variation over the years, particularly before, during, and after the upgrade construction (Figure 5). Above and below average annual rainfall was recorded from 2012-2014 during the baseline surveys (Figure 5). Among these, 2014 was the driest year, with a recorded rainfall of 978 mm. The construction phase (2015-2018) also experienced some dry years, especially in 2016 (902mm). Subsequently, during the operational phase monitoring (2018-2023) the driest rainfall on record was recorded during 2019 (637mm) which was followed by two

exceptionally wet years (2021 and 2022). Of these, 2022 emerged as the wettest year on record, with a total rainfall of 2889 mm. In 2023, the months from January to April experienced dry conditions, with each month recording below-average rainfall.

The number of heavy rainfall days (>75 mm) followed a similar pattern to that of annual rainfall. Between 2012 and 2019, there were only a few or a single heavy rainfall day(s), which generally corresponded to below (2012, 2014, 2016, 2018, 2019) or slightly above average (2013, 2015, 2017) annual rainfall. In contrast, in 2021 there were five heavy rainfall days that contributed to the potential for flooding at Upper Warrell Creek. In 2022, there were ten days with more than 75 mm of rain. This increased the frequency and intensity of flooding events at Upper Warrell Creek. No rainfall events exceeding 75mm have been recorded in 2023.

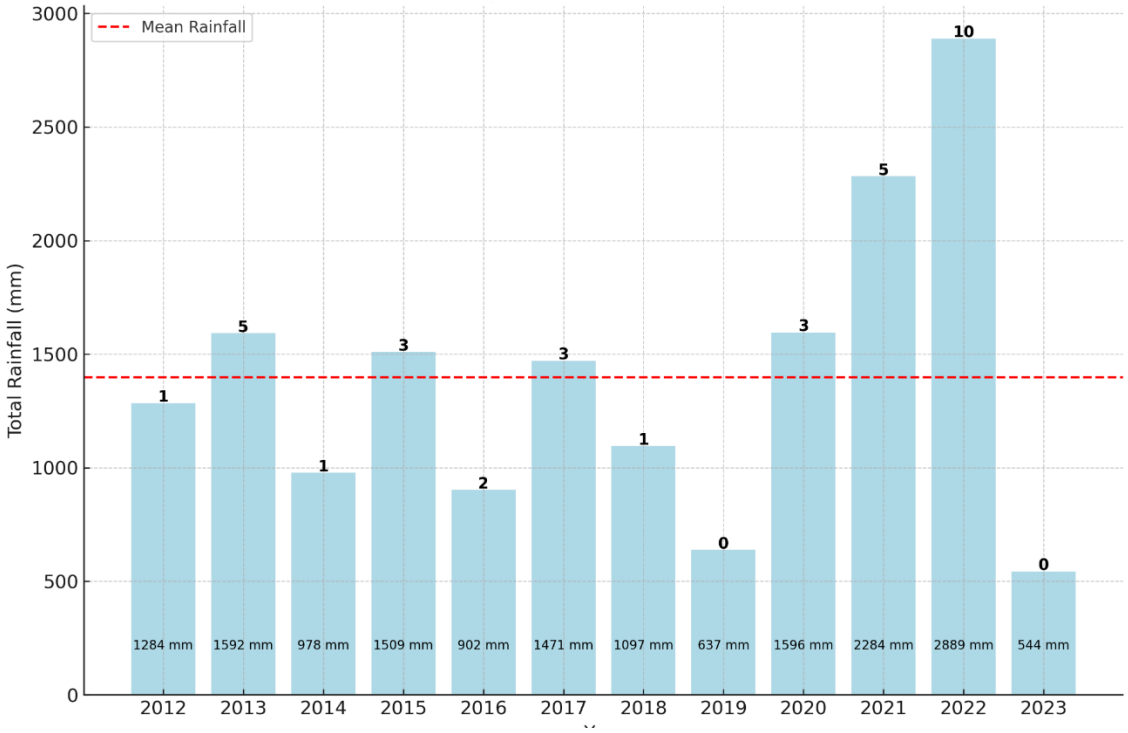

Figure 5: Total annual rainfall at the Bellwood weather station from 2012 to 2023. Numbers above bars represent number of likely flood events (i.e. >75mm in 24 hours). Red line = average annual rainfall (1399mm). Note 2023 = to date only as of 30 August 2023.

3.3 Tadpole surveys

No giant barred frog tadpoles or any tadpoles were detected at Upper Warrell Creek during any of the operational monitoring (Years 1, 3 and 5) dip-netting and bait trap surveys. The only vertebrate species captured were various freshwater fish. These included empire gudgeons (*Hypseleotris compressa*), striped gudgeons (*Gobiomorphus australis*), and australian smelt (*Retropinna semoni*). These species were recorded through both dip-netting and bait-trapping methods. Other records included various species of invertebrate fauna including freshwater shrimp.

3.4 Habitat assessment

The Upper Warrell Creek study area included a diverse range of habitats, ranging from grassland to moderate quality riparian and wet sclerophyll forest with a dense litter layer. The riparian forest was fragmented and

grazed in some parts, while the remainder consisted of a narrow strip of vegetation along the creek, surrounded by agricultural land. The riparian vegetation width varied, but it was mostly confined to the bank and did not exceed 30m. Leaf litter cover was high (>75%) in intact riparian zones, but low (<40%) in cleared and grazed areas. The creek bank topography also varied, with steep banks on both sides downstream of the alignment (Zones 1-6), and on the north bank upstream of the alignment (Zones 11-13). A flatter bank profile occurred on the north bank near the alignment (Zones 7-11), and upstream (Zones 14-21).

3.5 Water quality

The surface water samples collected from Upper Warrell Creek during summer and autumn 2023 showed that the pH of both samples was below the ANZECC guidelines, indicating slightly acidic conditions (Table 2). The dissolved oxygen levels were also below the ANZECC guidelines (Table 2). Heavy metal concentrations were low, and nutrient levels were below the ANZECC guidelines for 95% protection level for lowland streams in south-eastern Australia (Table 2). Notably, there were no indications of hydrocarbon contamination (Table 2).

Comparing the two samples, the autumn sample had a lower temperature and slightly lower pH than the summer sample. Additionally, the autumn sample exhibited increased turbidity and higher levels of dissolved oxygen compared to the summer sample.

Table 2: Water quality parameters for the summer and autumn samples in 2023 at Upper Warrell Creek in comparison to the ANZECC guidelines (95% protection) for lowland streams in south-east Australia (ANZECC 2000). ORP = oxidation-reduction potential. NTU = Nephelometric turbidity units. TDS = Total dissolved solids. DO = Dissolved oxygen. ID = insufficient data to derive a reliable trigger value.

Parameter (unit)	Summer 2023 sample	Autumn 2023 sample	ANZECC Trigger (95% species level protection)
General parameters			
Temperature (°C)	27.91	15.02	N/A
pH (pH units)	6.23	6.12	6.5-8.0
ORP (mV)	125	265	N/A
Conductivity (dS/m)	0.247	0.262	125-2200
Turbidity (NTU)	8.3	7.1	2
Dissolved Oxygen (mg/L)	0.9	4.49	9-10.5
DO% Saturation (%)	11.7	46	80-110
TDS (g/l)	0.161	0.17	N/A
Salinity (parts per thousand (ppt)	0.1	0.1	N/A
Hydrocarbons			
Benzene (µg/L)	<1	<1	ID
Toluene (µg/L)	<1	<1	ID
Ethylbenzene (µg/L)	<1	<1	ID
m+p-xylene (µg/L)	<2	<2	ID
o-xylene (µg/L)	<1	<1	ID
Naphthalene (µg/L)	<1	<1	ID
TRH C6 - C9 (µg/L)	<10	<10	ID
TRH C6 - C10 (µg/L)	<10	<10	ID
TRH C6 - C10 less BTEX (F1) (µg/L)	<10	<10	ID
TRH C10 - C14 (µg/L)	<50	<50	ID
TRH C15 - C28 (µg/L)	<100	<100	ID
TRH C29 - C36 (µg/L)	<100	<100	ID
TRH >C10 - C16 (µg/L)	<50	<50	ID
TRH >C10 - C16 less Naphthalene (F2) (µg/L)	<50	<50	ID
TRH >C16 - C34 (µg/L)	<100	<100	ID
TRH >C34 - C40 (µg/L)	<100	<101	ID
Surrogates			

Parameter (unit)	Summer 2023 sample	Autumn 2023 sample	ANZECC Trigger (95% species level protection)
Surrogate Dibromofluoromethane (%)	110	96	No guideline
Surrogate toluene-d8 (%)	104	91	No guideline
Surrogate 4-BFB (%)	98	97	No guideline
Surrogate o-Terphenyl (%)	61	74	No guideline
Heavy metals			
Silver-Total (µg/L)	<1	<1	0.012
Aluminium-Total (µg/L)	50	<10	552
Arsenic-Total (µg/L)	2	<1	13.2
Cadmium-Total (µg/L)	<0.1	<0.1	0.22
Chromium-Total (µg/L)	<1	<1	1.2
Copper-Total (µg/L)	<1	<1	1.42
Iron-Total (µg/L)	1200	700	1000
Manganese-Total (µg/L)	410	61	1900
Nickel-Total (µg/L)	1	<1	11.2
Lead-Total (µg/L)	<1	<1	3.4
Selenium-Total (µg/L)	<1	<1	11.2
Zinc-Total (µg/L)	1	<1	8
Mercury-Total (µg/L)	<0.05	<0.05	0.06
Nutrients			
Phosphorus - Total (mg/L)	<0.05	<0.05	0.05
Total Suspended Solids (mg/L)	14	15	25
Phosphate (mg/L)	<0.005	<0.005	0.025
Nitrite (mg/L)	<0.005	0.03	0.9
Nitrate (mg/L)	0.006	<0.005	1
Ammonia (mg/L)	0.036	0.046	Dependent on pH
Total Nitrogen in water (mg/L)	0.2	0.2	0.5

3.6 Giant barred frog surveys

3.6.1 Age classes and abundance

During operational phase monitoring at Upper Warrell Creek (Years 1, 3, 4, and 5), a total of 66 giant barred frogs were captured or audibly detected (see Appendix A, Table A1; Table 3). Of these, 55 were considered distinct individuals: 52 recorded during the operational phase and three initially tagged during the construction phase (See section 3.5.2). Among these, 48 were verified through physical capture, while the remaining seven were identified as calling males but were included in the total individual count (see Appendix A, Table A1).

Adults made up the majority of records, accounting for 64% (42 out of 66, Table 3). The adult population was divided into 16 females and 26 males, representing approximately 24% and 39% of the overall count, respectively. Sub-adults, characterised by snout-vent (S-V) lengths between 40 and 60mm, constituted 24% of the records (16 out of 66). The remaining 12% were juveniles with S-V lengths under 40mm. Female adults had S-V lengths ranging from 79.5 to 119mm, while males ranged from 63 to 83mm (Table 3).

During operational monitoring variations were observed in the composition of age classes (Table 3). Year 1 recorded 12 records, primarily adults, including 7 males and 4 females, along with a single sub-adult. In Year 3, the count increased to 21 and featured a more diversified age distribution: 7 adult males, 2 adult females, 3 juveniles, and 9 sub-adults. Year 4 saw a peak of 25 records, with numbers distributed equally between age classes and sexes, while Year 5 experienced a decline to just 8 records, five adults and one juvenile (Table 3).

Seasonal fluctuations were also evident (Table 3). Spring had the fewest records, totaling 17, and was mainly dominated by adults. Summer, with 24 records, showed a balanced age distribution, particularly in Year 4. This contrasted with Year 1 and Year 3, where only adults were recorded in Year 1, and juveniles and sub-adults

were more prevalent in Year 3. Autumn had the highest count with 25 records, and was notable for a larger number of sub-adults, especially in Years 3 (6 records) and 4 (4 records).

Shifting from the baseline to the Year 1 construction phase led to a sharp decline in the number of recorded frogs (Figure 6). Specifically, 47 detections in the baseline year fell to just 16 in the first year of construction. This decline occurred for both new individuals, which decreased from 38 to 14, and recaptures, which dropped from 9 to 2 (Figure 6). No indiviuals from the baseline sample were recaptured during construction.

In contrast, the operational phase initially started to reverse this downward trend (Figure 6). The number of total captures rose from 12 in the first year to 21 in the third year. This increase was largely driven by new individuals, which reached 18 in year 3, while recaptures decreased to only 3 (Figure 6).

Subsequently, the increase in total detections peaked in the fourth operational year when 25 giant barred frogs were recorded (Figure 6). New individuals accounted for 20 of these, and recaptures contributed 5. However, this positive trend was not sustained in Year 5, which saw a drop to just 8 new individuals and, notably, no recaptures.

Table 3: Temporal comparison in the age classes of giant barred frogs recorded at Upper Warrell Creek during operational phase monitoring. Records include individual captured and audibly detected (Males only).

Year	Season	Adult		Immature		Seasonal total
		Female (79.5-119mm)	Male (63-83mm)	Juvenile (<40mm)	Sub-adult (40-60mm)	
Year 1	Spring	1	1			2
	Summer	1	3			4
	Autumn	2	3		1	6
Year 3	Spring	1	2			3
	Summer		1	2	3	6
	Autumn	1	4	1	6	12
Year 4	Spring	4	4			8
	Summer	4	3	2	2	11
	Autumn	1	1		4	6
Year 5	Spring	1	3			4
	Summer		1	2		3
	Autumn			1		1
Total		16	26	8	16	66

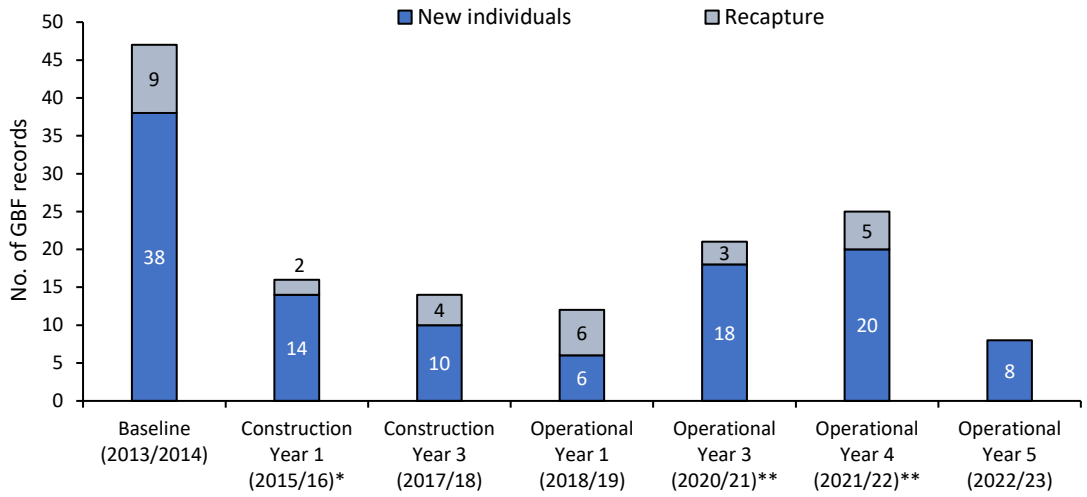

Figure 6: Total count of giant barred frog records, including new individuals and recaptures, throughout the baseline, construction, and operational phase monitoring at Upper Warrell Creek, WC2NH 2023. * Year one construction phase number may be an underestimate as it does not include frogs recorded in autumn 2015 (GeoLink 2018). **could include recapture of unmarked sub-adults.

3.6.2 Recaptures

During operational monitoring, a total of eight giant barred frogs were identified as recaptured individuals (**Error! Reference source not found.**). These recaptured individuals were verified using pit tags (6 individuals) and dorsal photos (2) (**Error! Reference source not found.**). Three of the individuals were originally tagged during the construction phase (GBF#2,3,4), with the remaining captured during operational monitoring.

Most of the recaptured frogs (5 out of 8) were only captured once. GBF#3 was captured four times, GBF#4 was captured three times, and GBF#2 was captured twice. GBF#3 was the only frog that was tagged before spring 2020 and recaptured afterwards (**Error! Reference source not found.**).

The locations of the PIT-tagged frogs along the transect were mapped to see if they crossed the alignment (Figure 7). The frogs usually stayed within a range of less than 100m from their initial capture location (Figure 7). Five frogs remained in the same zone, while three frogs moved to different zones. Two of these frogs (GBF#4 and GBF#3) moved to adjacent zones (Zones 4-5 and Zones 5-6, respectively, Table 4). One frog (GBF#2) crossed the alignment and travelled about 800m upstream from Zone 2 to Zone 20 (Table 4 and Figure 7).

Table 4: Recapture data of giant barred frog individuals during operational phase monitoring at Upper Warrell Creek. Phases: Con = construction, Op = Operational. ID = pit tag number.

Frog ID (GBF#)	Count	Verification technique	Initial capture date & phase	Recapture dates	Capture zones
GBF#2	2	Pit tag (ID: 00078ABB9B)	02/07/18 (con)	07/02/18, 17/10/18	3
GBF#3	4	Pit tag (ID: 00077E8FEF)	11/06/17 (con)	26/02/19, 20/03/19, 27/10/20, 17/11/2021	5 and 6
GBF#4	3	Pit tag (ID: (00078ABBF2)	07/05/18 (con)	05/02/18, 26/02/19, 27/10/20	4 and 5
GBF#6	1	Pit tag (ID: 991001000620121)	19/03/19 (Op)	28/10/2020	2 and 20
GBF#23	1	Pit tag (ID:956000010433901)	15/04/21 (Op)	17/11/2021	5 and 6
GBF#25	1	Dorsal photo	15/04/21 (Op)	18/11/2021	4 and 7
GBF#26	1	Dorsal photo	15/04/21 (Op)	18/11/2021	4 and 5
GBF#29	1	Pit tag (ID:960000011459761)	18/11/21 (Op)	9/02/2022	4

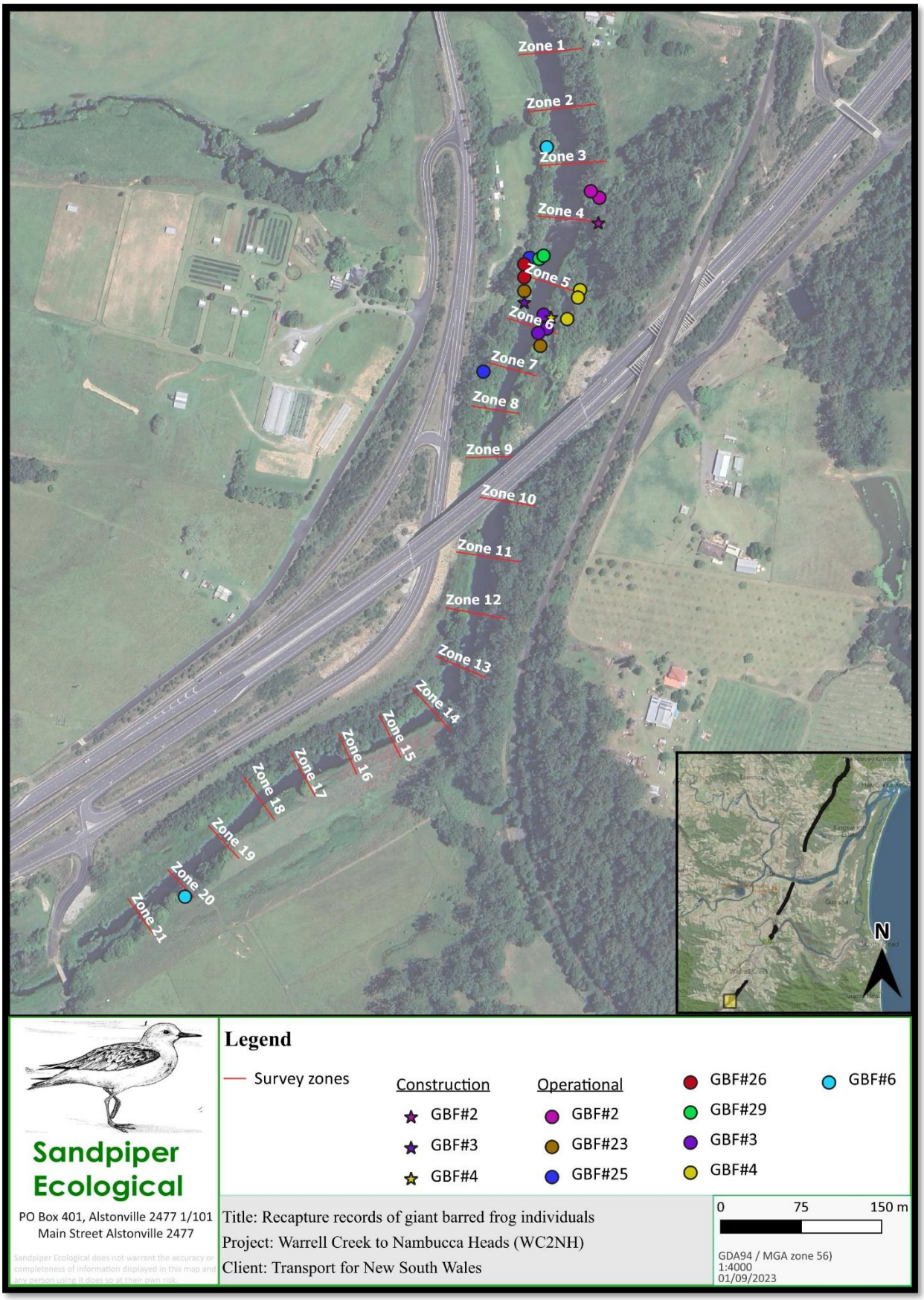

Figure 7: Location and movement patterns of recaptured giant barred frogs (individuals) recorded during the operational phase monitoring at WC2NH. The map includes both initial capture locations including three individuals that were originally tagged during the construction phase. Unique colours represent individuals.

3.6.3 Spatial distribution

At Upper Warrell Creek, all of the 66 giant barred frog detections occurred within 10 meters of the creek's edge, with 71.2% (47 records) found within 6 meters of the waters edge (Appendix A, Table A1). The primary bank within the riparian forest was the preferred habitat. Within this area 27 frogs were located on exposed leaf litter, while 17 were found in leaf litter with cover, such as beneath a lomandra or at the base of a tree. Leaf litter microhabitats accounted for two-thirds (66.6%) of all records (see Appendix A, Table A1). Notably, in the aftermath of flooding events during years 3 and 4, frogs were captured on bare earth (6 records) or on bare earth with partial vegetation cover (7 records).

During the operational monitoring in years 1, 3, 4, and 5 at Upper Warrell Creek, specific survey zones showed clustering of giant barred frog detections (Figure 8 and Figure 9). Zones 3 through 8 were particularly noteworthy for the clustering of records throughout operational monitoring (Figure 8 and Figure 9). Among the downstream zones, zone 5 recorded the highest number of decteions with 14 following by zone 4 (13) and zone 8 (6) (Figure 8). In total, the downstream areas contributed 49 records, comprising approximately 74.2% of all detections. Upstream, zone 13 was most active with 5 detections, while zones 12, 16, and 20 each recording one frog. Seventeen records (25.8%) were recorded upstream of the highway. Interestingly, zone 9, intersected by the alignment, and zones 1 (downstream), 14, 15, and 21 upstream did not record any giant barred frogs (Figure 8 and Figure 9).

During the operational phase monitoring, spatial and temporal data showed distinct trends in frog distribution. In year one, most frog detections occurred on the northern bank downstream of the highway (Figure 9). In years three and four, frog numbers increased in two areas: first, on the southern bank between hydrological zones 4-9 (downstream); second, in the riparian forest between zones 11 and 13 (upstream). By the end of the monitoring period, eight frogs were evenly distributed across both upstream/downstream and northern/southern banks.

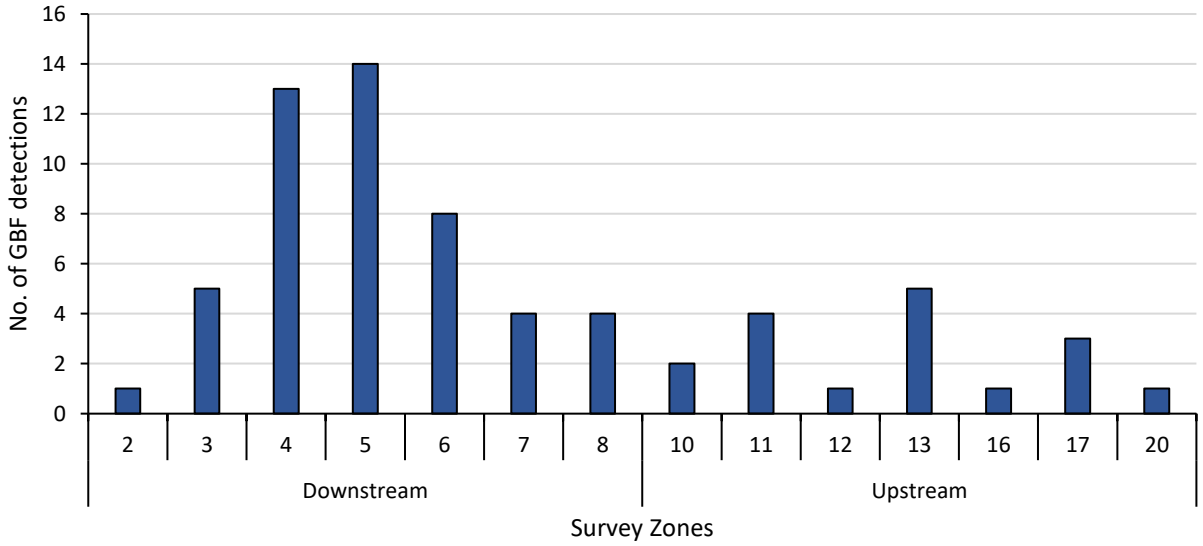

Figure 8. Number of giant barred frog records in relation to the survey zones downstream and upstream of the alignment. Note the alignment passed directly through zone 9. No individuals were recorded in zones 1, 9, 14, 15 and 21 upstream and were removed.

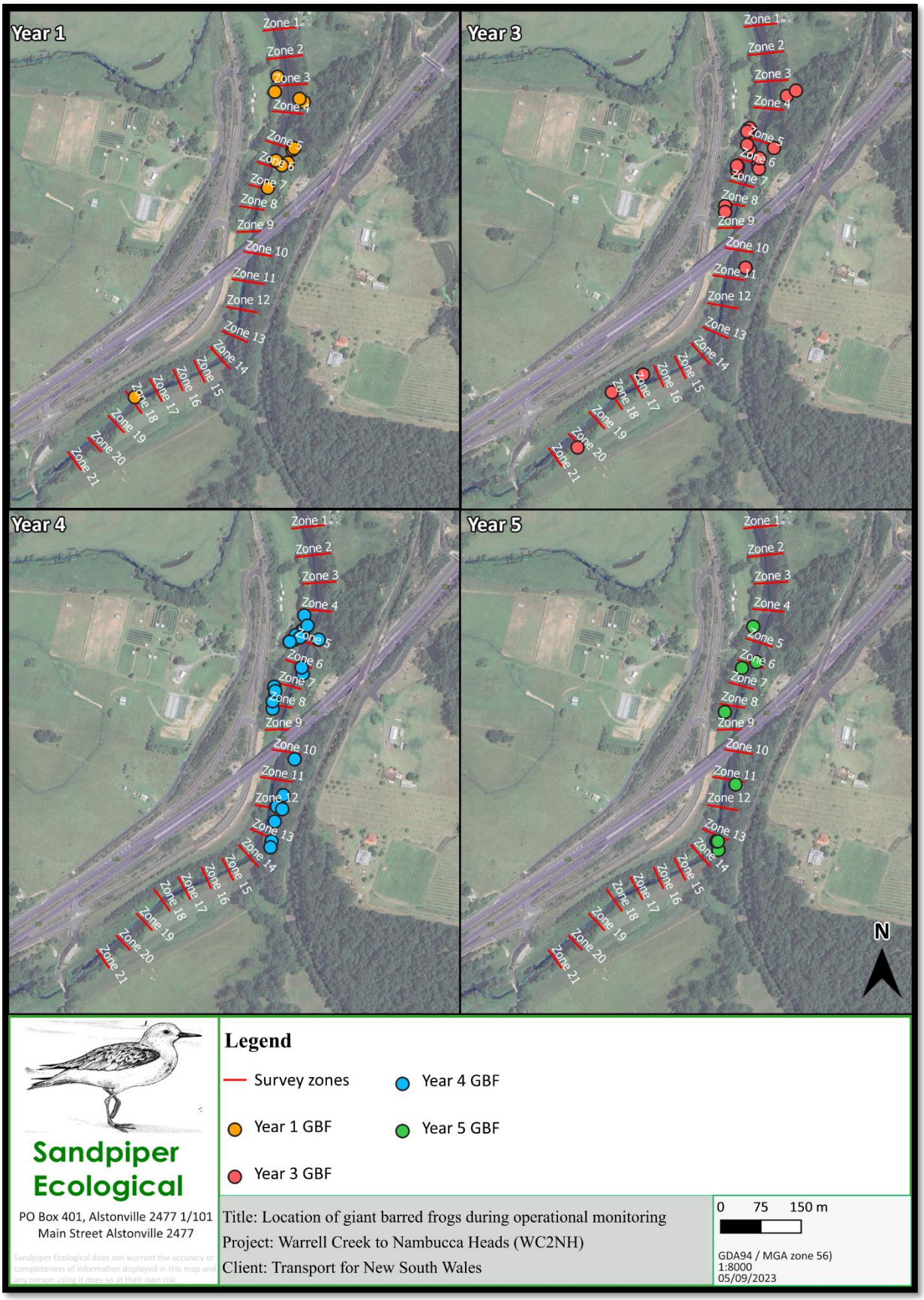

Figure 9: Location of giant barred frog records during year 1,3,4 and 5 of operational phase monitoring at Upper Warrell Creek.

3.6.4 Population estimates and trends

A comparison of adult population estimates across the seven 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 5 and Figure 10). The population estimate of 43 adult frogs (CI 26.6) in 2013/14 declined to seven (CI 9.77) in year one of the construction phase with estimates of eight (CI 10.46) and seven (CI 4.8) recorded in year 3 construction phase and year one operation phase respectively (Table 5 and Figure 10). The population increased substantially in years three and four of the operational phase with population estimates of 19 (CI 21.46) and 21.5 (17.38) adult frogs respectively. This was followed by a decline to only 3 individuals in year 5 monitoring (Table 5). Notably, a precise population estimate and confidence interval could not be determined for the year 5 data due to the absence of recaptures.

Table 5: Population estimates of adult giant barred frog at Upper Warrell Creek prior to construction (Lewis 2014), during construction (GeoLink 2018) and operational phase (Sandpiper 2019-2023).

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)	Year 5 OP (2022/2023)
GBF population estimate	43	7	8	7	19	21.5	3
95% confidence interval	26.6	9.77	10.46	4.8	21.46	17.38	N/A

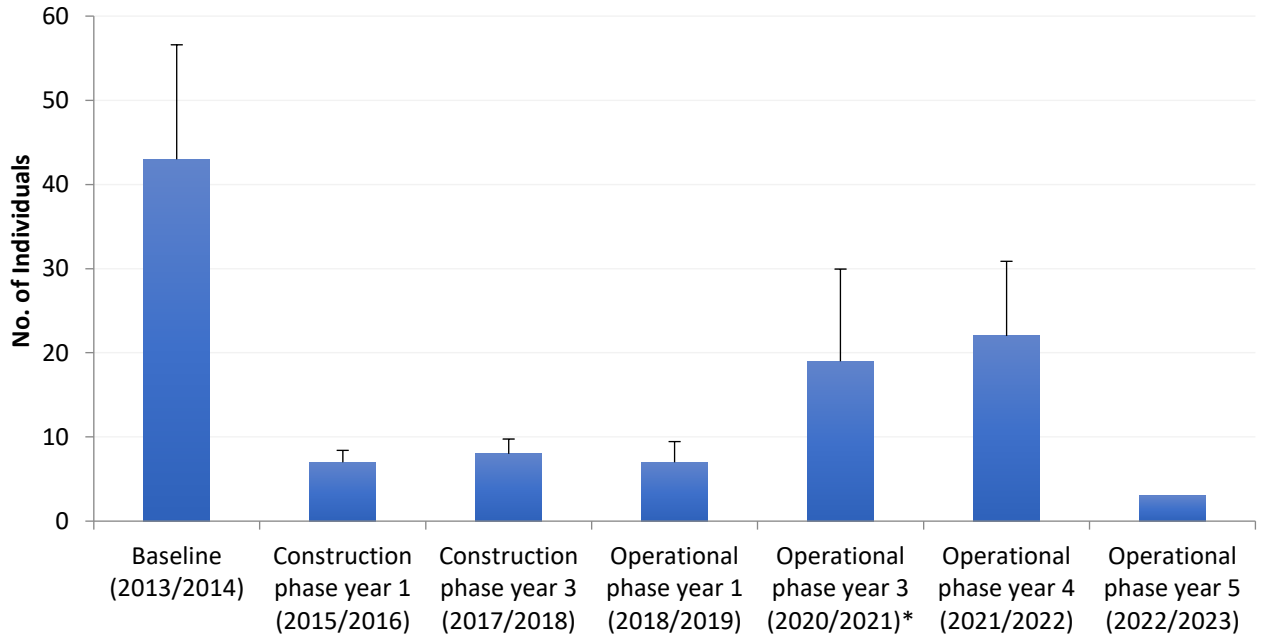

Figure 10: Adult population estimates (+ standard error) at Upper Warrell Creek during baseline (Lewis 2014b), construction phase (GeoLink 2018), year one operational phase (Sandpiper Ecological 2019), year three operational phase monitoring (Sandpiper Ecological 2021) and year four operational phase (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. Note an accurate population estimate and SE could not be derived for year five as no recaptures were recorded.

3.6.5 Chytrid sampling

Analysis of swabs from year four monitoring 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) 2022. 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).

3.7 Rainfall and giant barred frog abundance

Giant barred frog records at Upper Warrell Creek tended to increase as rainfall increased. The linear regression analysis revealed a significant positive relationship (n=12,F=6.48, p-value = 0.029) between seasonal rainfall and the number of giant barred frog captures (Figure 11 and Appendix B, Table B1). An R-squared value 0.393 suggests that 39.3% of the variation in the number of frog captures can be explained by the seasonal rainfall (Appendix B, Table B1). The scatter plot substantiates these findings, showing a trend where seasons with higher rainfall generally corresponded to a higher number of frog captures (Figure 11).

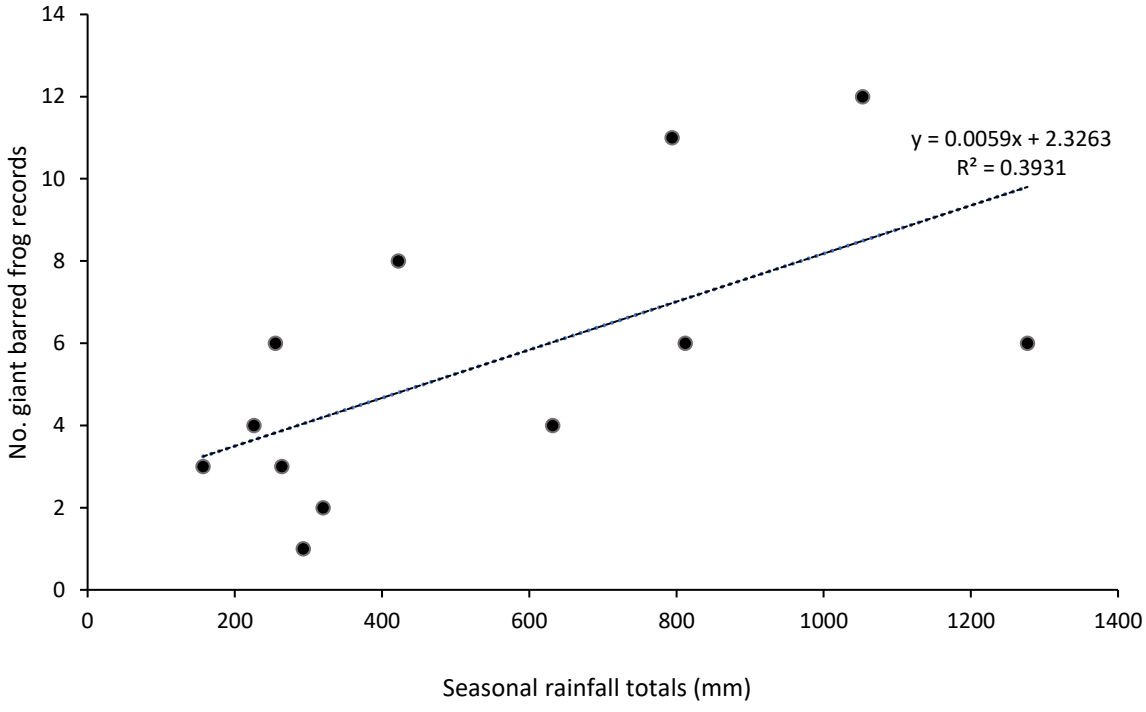

Figure 11. Linear regression between the number of giant barred frog recorded and seasonal rainfall at Upper Warrell Creek. Note that the R squared value of 0.393 indicates that rainfall explains approximately 39.3% of the variation in frog captures (Appendix B, Table B1).

4 Discussion

As outlined in the GBFMS, the primary aim of population monitoring is to assess if mitigation measures have preserved or enhanced the population size and habitat of the giant barred frog at Upper Warrell Creek. To determine this, the baseline, construction, and operational data will be compared and the key findings of operational monitoring will be discussed in relation to relevant literature. The main outcomes will then be summarised and evaluated based on the potential indicators of success specified in the GBFMS. From this, an evaluation on the effectiveness of the mitigation measures and whether the aim has been achieved will be determined with recommendations included.

The giant barred frog population at Upper Warrell Creek has fluctuated between 2013 and 2023. Starting with an estimated 43 individuals in 2013/14 (Baseline survey), the population experienced a sharp decline during the construction phase, reaching estimates of seven and eight individuals in the first (2015/16) and third years (2017/18), respectively. Notably, the third (2020/21) and fourth years (2021/22) of the operational phase monitoring saw a recovery, with the estimated population size increasing to 19 and 21.5 individuals. However, this recovery was short-lived, as year five (2022/23) recorded a substantial decline to an estimated three individuals. Population fluctuations appear characteristic of giant barred frogs, as similar patterns have been observed at other monitoring sites along the Pacific Highway (Sandpiper Ecological 2022a, Niche 2023 and Lewis 2018). Population fluctuations have been consistent at both impacted sites, where the highway passes through giant barred frog habitat, and reference sites, where the highway has no direct impact. Comparable variation was observed at Greys Dam (impact), and Arrawarra Creek (reference) at Sapphire to Woolgoolga (S2W; Sandpiper Ecological 2022a), Cooperbung Creek (impact and reference), Pipers Creek (impact and reference), Maria River (impact) and Smiths Creek (impact) at Oxley Highway to Kempsey (OH2K; Niche 2023) and four impact and four reference sites at Woolgoolga to Ballina sections 1 and 2 (W2B S1&2; Lewis 2018).

To understand these fluctuations, examining the concept of meta-populations is useful. Frogs, particularly along Australia's east coast, often exist in meta-populations created by habitat fragmentation and environmental conditions (Alford & Richards 1999). Sites along the Pacific Highway, such as Upper Warrell Creek, occur within a landscape mosaic with agricultural areas interspersed by patches of favourable riparian habitat, making it a prime example where frog meta-populations are likely to exist. These populations experience periods of decline, followed by times of high recruitment when environmental conditions are favourable. The recruitment success and population size depend on factors such as climate, floods, population age, habitat connectivity, and levels of disease (Green 2003; Newell 2018). Given the inherent variability of a meta-population, a number of factors can be expected to influence population numbers at sites like Upper Warrell Creek, irrespective of the highway construction.

4.1 Climate and hydrological conditions

Climate conditions, particularly rainfall, is one key factor influencing barred frog abundance through breeding and recruitment success (Knowles *et al*. 2015). Population estimates at Upper Warrell Creek largely mirrored rainfall trends during the construction and operational phase monitoring. The linear regression analysis revealed a significant positive relationship ($n=12, F=6.48$, p-value = 0.029) between seasonal rainfall and the number of giant barred frog captures. The first two years of construction and the initial year of operation (2015-2019) experienced average to below-average rainfall, and the population was estimated to be between seven and eight individuals during this period. These drier conditions were characterised by a higher proportion of adult frogs, suggesting low recruitment due to limited breeding opportunities. Interestingly, the number of giant barred frogs also declined at OH2K during this period, with as much as a 100% decline seen at the Cooperbung reference and impact sites (Niche 2023). Above-average rainfall in 2020-2021 led to a noticeable population recovery at Upper Warrell Creek, with the population increasing from eight individuals

in year one to 19 in year three (2020-21) and peaked at 21.5 in year four (2022-23). Notably, sub-adult frogs and juveniles comprised up to 63% of the population in year three. Wet years typically increase the number of breeding opportunities and, consequently, the recruitment success of barred frog species (Newell *et al.* 2013; Knowles *et al.* 2015).

Furthermore, a noticeable rise in invertebrate abundance, the primary food source for the giant barred frog (Lemckert & Shoulder 2007), was observed in 2022 (Sandpiper Ecological 2022), likely due to the favourable climatic conditions. Together, these factors created suitable conditions for the breeding and growth of the giant barred frog population at Upper Warrell Creek. The presence of gravid females, calling males, and males with dark nuptial pads, especially in year four, are indicative of active breeding in response to good conditions.

Flooding presents another factor contributing to the movement of frogs in and out of the study area, as discussed in the year three and four monitoring reports (Sandpiper Ecological, 2021 and 2022). Confirming the influence of flooding requires repeated sampling over consecutive years both downstream and upstream of study area, which is beyond the scope of the monitoring program. However, several lines of evidence suggest a tangible effect of floods on the giant barred frog population:

1. Change in the spatial distribution of frogs: Prior to the December 2020 flood events, zones 4-7 on the northern creek bank recorded the highest number of giant barred frog captures during both the construction and year one operational phase. Distinguished by low elevation, these areas were prone to flood-induced alterations to habitat quality. Post-flooding habitat assessment in year three monitoring showed accumulation of flood debris, creek bank erosion, and depletion in leaf litter and ground-level vegetation. Data collected from the third and fourth years of monitoring identified a discernible shift in the spatial distribution of giant barred frogs. The newly observed concentrations occurred in zones 4-8 along the southern creek bank and zones 11-13 on the northern creek bank—areas characterised by steeper bank profiles likely acting as a refuge from flood waters (Lewis 2014b).

2. Variation in recapture rates: The recapture rates were high during the initial construction and operation phase between 2015-19, which had moderate to below-average rainfall and infrequent flood events. The average recapture rate between seasons in the first operational year was 52.7% (Sandpiper Ecological, 2019). However, recapture rate decreased in the following years (2020-23), along with increased rainfall and flooding frequency and severity. Notably, the recapture rates during the year three and year four monitoring events dropped below 10%, starkly contrasting the rates observed in the first year of operational monitoring (Sandpiper Ecological 2019). After multiple flood events from 2020 through spring 2022, no recaptures were observed during year five of monitoring with several new individuals arriving. This sharp decline in recaptures and increase in new individuals, particularly following flood events, suggests that flooding may influence frog movement into and out of the study area.

3. Flood severity and new observations: The catchment area experienced increasing rainfall totals from 2020 to 2022, with well-above-average rainfall recorded in 2022. This likely led to the saturation of the catchment area, which, combined with several heavy rainfall days (10 days >75mm in 2022), increased the intensity and frequency of floods. The floods changed the Upper Warrell Creek habitat, by scouring the ground, depositing flood debris, and causing bank erosion resulting in tree fall. Previous studies have shown that juvenile frogs are naturally vulnerable to flood-mediated displacement because of their size (Koch & Hero 2007). However, the third-year post-flood monitoring in 2021 also noted the presence of several large gravid adult frogs that had not been recorded before, in addition to juveniles and sub-adults. Considering the high recapture rates recorded in year one operation monitoring (around 50% on average) and the estimated detection rates of 0.54-0.65 across three surveys for giant barred frogs (Lollback 2021), coupled with their known lifespan of 5-6 years (Lewis 2014b), it is unlikely that all of these new gravid females (N=five) went undetected in previous surveys. This suggests that some adult frogs were

transported to the area during floods and equally, some adult frogs would have been washed out of the study area during this period.

The possibility that frogs moved into or out of the sample population during the sample period raises concerns about the validity of the population estimate. A key assumption of the population estimate procedure is limited immigration, emigration, and mortality during the sample period (Fowler *et al*. 1999). This implies that the population estimate may be biased or inaccurate. For instance, net immigration due to flooding would inflate population estimates, whereas net emigration would result in deflation. Although flooding could theoretically balance immigration and emigration, data indicate a net loss of individuals in year five. This is substantiated by six heavy rainfall events (>75mm) between the conclusion of year four monitoring in April 2022 and the initiation of year five monitoring in December 2022.

The study area's riparian zone warrants further consideration. Characteried by its narrow, degraded, and fragmented condition, this environmental context likely intensifies flood impacts on the frog population. Specifically, the degraded riparian zone restricts refuge habitats and hampers along-bank movement upstream of the highway. Such limitations could skew the balance between immigration and emigration during flood events, adding another layer of complexity to population estimate interpretations.

4.2 Chytrid fungus

In addition to climatic variables, the year four monitoring report discussed another important variable affecting frog populations: chytrid fungus (Sandpiper Ecological 2022). Chytrid fungus (*Batrachochytrium dendrobatidis- bd*) has been confirmed in the Upper Warrell Creek frog population and poses a potential threat to its stability. Chytrid fungus causes a disease called Chytridiomycosis, which can have various outcomes for amphibians, ranging from rapid population decline to possible recovery and resistance after infection (Lips2016; Newell *et al*. 2013; Retallick *et al*. 2004). The presence of chytrid fungus in the Upper Warrell Creek frog population was confirmed during the baseline and construction surveys, with four out of 22 tested frogs showing positive results (Lewis 2014b; Geolink 2018). One of these frogs tested negative in a later sample, indicating recovery from the infection (Geolink 2018). However, the year four monitoring data showed a an increase in chytrid detections, with five confirmed and six possible cases, resulting in a potential prevalence rate of 44% among the sampled frogs (Sandpiper Ecological 2022). This could explain the observed declines in frog numbers during year five monitoring, as chytrid-related population crashes have been documented in other studies (Retallick *et al*. 2004; Penman *et al.* 2008).

No obvious symptoms of chytrid infection, such as lethargy or discoloration, have been reported at Upper Warrell Creek, however the pathogen's impact should not be overlooked. Moreover, previous research has found that juvenile giant barred frogs are more likely to be infected with chytrid fungus than adults, and that infection intensity is negatively correlated with snout-vent length (Kriger 2006). This suggests that the successful recruitment of juveniles and sub-adults recorded during year three and four monitoring may have been compromised by the high prevalence of chytrid fungus in year four. Furthermore, environmental conditions such as lower temperatures and increased rainfall can influence the infection dynamics of chytrid fungus, as they are associated with higher Bd prevalence, infection intensity, and rates of gaining infection (Holanders *et al*. 2022). These conditions were experienced during 2021-2022, particularly between the end of year four and the beginning of year five monitoring periods (i.e. April-October 2022), which may have increased the susceptibility of the younger cohort at Upper Warrell Creek. Interestingly, chytrid fungus was detected in the frog population even before any construction activities took place, indicating that it was likely introduced prior to the commencement of monitoring. However, understanding the complex interactions between frog population age structures, environmental conditions and chytrid fungus is challenging, and it is difficult to assess the severity of its impact on the Upper Warrell Creek population. Nevertheless, the elevated incidence of chytrid fungus observed in the fourth year, coupled with the subsequent decline in population

and absence of recaptures in Year five, implicates the fungus as a potential contributory factor to population decline, particularly under cooler *La Nina* conditions.

4.3 Habitat modificiation – highway construction

The year three operational monitoring report detailed habitat changes as a result of highway construction activities at Upper Warrell Creek (Sandpiper Ecological 2021). Aerial photographs from 2010 and 2013 were used as historical benchmarks to compare stream morphology before and after construction. The photographs revealed that geomorphic features such as back channels, lateral bars, and fluvial islands existed in the pre-construction period. These features offered high-quality breeding habitats for giant barred frogs by providing refuge from predators and buffering against hydrological fluctuations (Lewis 2014a). However, the photographs were captured during high-rainfall events, which might have skewed the baseline hydrological conditions.

Lewis (2014b) identified Zones 8 and 9 as ecologically vital for giant barred frogs because they contained geomorphic features that supported their breeding behaviour. In the baseline sample 44% of frogs were found in Zones 8 and 9, indicating the ecological importance of these zones within the 1 km sample area. However, construction activities, especially bridge construction, altered the stream morphology of both zones. For instance, the back channel of Zone 8 was partially eliminated by the reshaping of the northern channel, reducing the availability of refuge habitats. Moreover, the upstream section of the back channel in Zone 8 was modified by the introduction of rock fill, which may have degraded its suitability as breeding habitat by changing its hydrological characteristics and removing the natural bank structure. A small back-channel on the north bank in zone 18 was also dominated by dense vegetation following removal of stock, likely rendering this area unsuitable for frogs.

Some potential breeding habitat remains within the study area, particularly the lateral bar on the north bank in zones 6 and 7. This area includes a shallower littoral zone with overhanging banks both on the main channel and back channel sides and is the only area where male frogs were consistently heard calling throughout the monitoring program. Lollback (2021) and Lewis and Rohweder (2005) studied the habitat requirements of giant barred frogs and suggested they prefer riparian forests with abundant leaf litter, sparse ground vegetation, and undercut banks in creeks with a pool/riffle sequence. Occupancy models revealed a higher probability of frog presence in habitat areas with ponds longer than 10m and undercut banks (Lollback 2021). Habitat at Upper Warrell Creeek differs to that studied by Lollback (2021) and Lewis and Rohweder (2005) as it lacked a pool/riffle sequence and contained a single riffle within the 1km study area. Interestingly, the highest abundance of frogs in the baseline was recorded just downstream of the riffle zone in an area that included a riffle, the start of a large pool and back-channel pools.

According to Knowles *et al.* (2015) giant barred frogs lay their eggs in the water and the female deposits the eggs on a near vertical or overhanging bank from a floating position. Whilst the subject site includes an abundance of vertical and overhanging banks, access to and from the water would be challenging and frogs floating in the water would be subject to predation from eels and predatory native fish. Predation risk on adult frogs at Upper Warrell Creek may be higher than at sites with smaller pools and riffles. Whilst there is approximately 400m of habitat with steep undercut banks the suitability of this habitat for breeding is uncertain. The restricted occurrence of calling males is indicative of suitable breeding habitat extent.

4.4 Distribution and Movement

During year five of operational monitoring at Upper Warrell Creek, several noteworthy patterns in giant barred frog distribution and habitat use were observed. While no individuals were found to have moved beneath the highway, the species was detected in five out of 21 survey zones. Continued occupation of several zones

suggests that the population persists in the study area, albeit with reduced spatial distribution compared to baseline surveys (Lewis 2014b). Interestingly, the fifth-year data again recorded individuals on the north bank within zones 11-13, a location where frogs had not been previously recorded in the first three years of the operational phase. This sudden clustering in these zones is attributed to flood-induced movement as suggested in years three and four (Sandpiper Ecological 2021, 2022).

The majority (74.2%) of giant barred frog detections throughout operational monitoring were concentrated in downstream zones, specifically zones 3 through 8, with zone 5 exhibiting the highest activity. This downstream preference has been a consistent pattern throughout the monitoring period. However, upstream zones were not devoid of activity, contributing to 25.8% of total detections, mostly within zone 13.

In terms of microhabitat preference, leaf litter remains the dominant substrate where two-thirds of the individuals were found, while a small proportion were found on bare earth, potentially due to the removal of leaf litter by flood events. Notably, all detections occurred within a 10-meter radius from the creek's edge, this coincides with 71.2% of records found within a 6-meter radius from the water's edge. These observations reinforce the importance of riparin forest on the primary bank as the species' preferred habitat.

Movement of giant barred frogs beneath the highway was confirmed during year three monitoring. A male frog, initially tagged in autumn 2019, was recaptured 880m upstream in spring 2020. Whilst giant barred frogs have been recording moving up to 200m in a night, average nightly movement distance is typically less than 25m (Lemckert and Brassil 2000). Records of highway crosses appeared to be more frequent at impact sites at OH2K where a total of twelve individuals crossed the alignment (Niche 2023). Movement at Upper Warrell Creek is likely hampered by dense pigeon grass (S*etaria sphacelata*) on the northern bank. This is discussed further in the following section.

4.5 Potential indicators of success

Continued presence of giant barred frog along any part of the 1 km transect

This indicator of success has been met. Despite experiencing population fluctuations giant barred frogs have persisted in various zones within the study area through all years of operational monitoring. Frogs were recorded in five out of 21 survey zones during year five of operational monitoring.

The recapture of one or more giant barred frog following their relocation from the clearing footprint (if this occurs); or the presence of tadpoles, metamorphs or juveniles frogs during follow up surveys post construction (LES 2014a).

Although no giant barred frogs were recaptured from the baseline surveys, juvenile frogs have been regularly observed during the operational phase. This data provides evidence of successful breeding and recruitment in the post-construction phase. Furthermore, the consistent detection of juveniles implies the existence of viable breeding habitats either within the surveyed transects or upstream. Such habitats are likely critical for sustaining the population amid current threats.

A <30% decline in measured habitat parameters; or <15% increase in bare ground cover;

Habitat parameters have remained relatively consistent during operational monitoring with the main change occurring post construction within zones 8 and 9. Previous monitoring reports have touched on potential habitat concerns. One notable aspect of concern was growth of pigeon grass (S*etaria sphacelata*) and broad-leaved paspalum (*Paspalum mandiocanum*) on the north bank in zones 5, 7 and 8. Pigeon grass also dominated the south bank of zones 10, 11, 19, 20 and 21. Dense grass represents a barrier to movement. Geolink (2015, 2018) recorded paspalum and/or pigeon grass in zones 7, 8 and 10, and images presented by Geolink (2018) show pigeon grass in zones 8 and 10. Based on available information, it seems likely that pigeon grass was

present at commencement of construction, however grazing by stock may have kept grass under control and the exclusion of stock, particularly on the south bank, has enabled grass to grow and form a barrier to movement.

Work to control pigeon grass and paspalum in zones 7, 8, 9 and 10 commenced in July 2021. The programmed works included targeted weed control aimed at reducing the extent / density of pigeon grass and paspalum followed by planting of 60 *Waterhousia floribunda* (on the northern bank in zones 7,8 & 9) to form a canopy and connect existing remnant canopy trees. Additional *Lamandra spp.* to bolster the plantings were also added into the area (Zone 9 on the northern bank under the bridge and as needed to repair flood damage). The scope of this work was developed in consultation with and endorsed by the EPA. Post-implementation, *Paspalum mandiocanum* was notably less prevalent in previously affected areas (Zones 7-9). Restoration efforts, which included planting *Waterhousia floribunda* in Zones 7-9 on the north bank, have been successful. However, for these plants to fully integrate with existing vegetation and counter weed spread, more time is necessary. Pigeon grass remains an issue in Zones 7 and 8 on the north bank.

During the 2021-2022 period, severe floods had a substantial impact on microhabitat. Numerous trees in the riparian zone were uprooted, and areas of grass and regrowth were either eroded or flattened. The floods also washed away leaf litter, leaving exposed earth in several areas of the riparian zone. Consequently, there was a decrease in litter cover, and a substantial increase in woody debris and scoured ground cover. However, the February 2023 habitat assessment revealed some positive improvements to microhabitat condition. There was a noticeable improvement in leaf litter cover, and scouring was less prominent. Flood debris were still present, particularly around the southern banks of Zones 4-8 and 11-13, creating potential refuge areas for giant barred frogs. The leaf litter and bare ground cover had returned to baseline levels and this indicator is considered to have been met.

No statistically significant changes in measured water quality parameters

Water quality analysis conducted during operational monitoring predominantly remained within the parameters established by ANZECC guidelines, thereby necessitating minimal concern. Results from year five monitoring showed a slightly low pH, reduced dissolved oxygen, and low levels of heavy metals, but no signs of hydrocarbon contamination (Table 2). These parameters could be influenced by low flow conditions. Stagnation can lower the dissolved oxygen and pH levels due to increased water temperature and higher biochemical oxygen demand (BOD) from the decomposition of organic matter (Lintern *et al.* 2018). However, the reliability of the water quality assessment is limited by the sampling approach, which only takes isolated samples from a single location at long intervals. This may not capture the spatial and temporal variations of water quality across Upper Warrell Creek (Leigh *et al*., 2019). As such, statistical analysis is not considered applicable and would require water sampling at a higher temporal and spatial resoluation to achieve valid comparisons (Leigh *et al*. 2019).

No road kill of Giant Barred Frog resulting from operation of highway.

This indicator has been met. No giant barred frogs have been recorded during construction or operational road kill monitoring.

5 Conclusion

Management of riparian habitat at Upper Warrell Creek aimed to either sustain or enhance the population and habitat of the giant barred frog. Monitoring of the giant barred frog population over the first five years of the operational phase indicates that most indicators of success have been achieved. Nonetheless, these indicators do not provide unequivocal evidence of a stabilised or improved population of the giant barred frog. This

uncertainty arises from the inherent variability in the frog's population, which is likely to have been influenced by an interplay of external variables such as climate conditions, flood events, disease outbreaks, and changes in habitat structure. As a result, the established indicators of success, in isolation, are inadequate to conclusively determine the effectiveness of the project in terms of long-term population stability or enhancement for the giant barred frog.

Of particular concern is the absence of recaptures and inability to accurately calculate a population estimate during year five. The removal of cattle from the site has enabled extensive grass growth, which would limit movement of barred frogs between riparian patches. Furthermore, construction work was centred on a morphologically complex part of the study area that included a riffle zone, with back channels and a lateral bar. Baseline surveys highlighted the importance of this are for giant barred frogs.

The monitoring program was hampered by the absence of a comparable reference site that would have provided comparative data to that collected in the study area.

6 Recommendations

Recommendations for future monitoring programs are provided in Table 6.

Table 6: Recommendations based on findings of operational phase giant barred frog monitoring program.

Number	Recommendation	Transport for NSW Response
1.	No further monitoring of giant barred frogs at Upper Warrell creek is required.	Agree
2.	Thorough consideration is required before initiating monitoring programs without inclusion of a suitable reference site.	Noted
3.	Greater consideration of baseline survey results and micro-habitat features is required during the planning phase of projects to reduce impacts on threatened species and ensure that remediation work is appropriate.	Noted

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Appendix A

Table A1: Operational phase monitoring field data for giant barred frog records at Upper Warrell Creek, 2018-2023.

Year	Season	Frog ID	GBF	No.	Date	Zone	Creek side	Distance from stream edge (m)	Distance ranges	Microhabitat	Sex*	Age*	S/V length (mm)	Weight (g)	Breeding condition*	Microchip ID	New or recapture	Chytrid detections
Year 1	Spring	Frog 1	GBF#	1	17/10/2018	5	North	3.4	2-4	Exposed leaf litter	Female	Adult	101.5	173.0	Gravid	991001000620130	New	N/A
Year 1	Spring	Frog 2	GBF#	2	17/10/2018	3	North	4.05	4-6	Exposed leaf litter	Male	Adult	77.1	67.0	Moderate	00078ABB9B	Recapture	N/A
Year 1	Summer	Frog 3	GBF#	3	26/02/2019	5	North	1.1	0-2	Vegetation cover	Male	Adult	83.8	85.0	Moderate	00077E8FEF	Recapture	N/A
Year 1	Summer	Frog 4	GBF#	4	26/02/2019	4	North	8.3	8-10	Exposed leaf litter	Female	Adult	101.5	141.0	Gravid	00078ABBF2	Recapture	N/A
Year 1	Summer	Frog 2	GBF#	2	26/02/2019	3	North	1.3	0-2	Leaf litter - cover	Male	Adult	74.8	76.0	Moderate-dark	00078ABB9B	Recapture	N/A
Year 1	Summer	Frog 5	GBF#	5	26/02/2019	3	South	NR	NR	N/A	Male	Adult	NR	NR	Calling	NR	New	N/A
Year 1	Autumn	Frog 5	GBF#	6	19/03/2019	2	South	1.54	0-2	Leaf litter - cover	Male	Adult	75.9	53.0	Moderate	991001000620121	New	N/A
Year 1	Autumn	Frog 6	GBF#	7	19/03/2019	17	South	5	4-6	Leaf litter - cover	Immature	Sub-adult	45.7	11.5	NA	NA	New	N/A
Year 1	Autumn	Frog 4	GBF#	4	20/03/2019	5	North	4.42	4-6	Exposed leaf litter	Female	Adult	99.5	165.0	Gravid	00078ABBF2	Recapture	N/A
Year 1	Autumn	Frog 7	GBF#	8A	20/03/2019	5	North	1.3	0-2	Leaf litter - cover	Male	Adult	73.2	57.0	Moderate	991001000620125	New	N/A
Year 1	Autumn	Frog 8	GBF#	8	20/03/2019	6	North	3.6	2-4	Leaf litter - cover	Female	Adult	92.5	116.0	Gravid	991001000620122	New	N/A
Year 1	Autumn	Frog 3	GBF#	3	20/03/2019	5	North	0.8	0-2	Vegetation cover	Male	Adult	81.8	85.0	Moderate	00077E8FEF	Recapture	N/A
Year 3	Spring	Frog 1	GBF#	3	27/10/2020	5	North	1.2	0-2	Leaf litter - cover	Male	Adult	83.7	85.0	Moderate	00077E8FEF	Recapture	N/A
Year 3	Spring	Frog 2	GBF#	4	27/10/2020	5	North	4	2-4	Exposed leaf litter	Female	Adult	98.7	141.0	Gravid	00078ABBF2	Recapture	N/A
Year 3	Spring	Frog 3	GBF#	6	28/10/2020	20	North	5	4-6	Exposed leaf litter	Male	Adult	75.3	58.0	Moderate	991001000620121	Recapture	N/A
Year 3	Summer	Frog 4	GBF#	9	17/02/2021	8	South	0.2	0-2	Exposed leaf litter	Immature	Sub-adult	51.4	15.0	N/A	NA	New	N/A
Year 3	Summer	Frog 5	GBF#	10	17/02/2021	6	South	0.7	0-2	Exposed bare earth	Male	Adult	NR	NR	Calling	NR	New	N/A
Year 3	Summer	Frog 6	GBF#	11	17/02/2021	6	South	4	2-4	Exposed leaf litter	Immature	Juvenile	36.1	5.0	N/A	NA	New	N/A
Year 3	Summer	Frog 7	GBF#	12	17/02/2021	16	South	3.5	2-4	Leaf litter - cover	Immature	Sub-adult	42.6	10.0	N/A	NA	New	N/A
Year 3	Summer	Frog 8	GBF#	13	17/02/2021	17	South	4.5	4-6	Exposed leaf litter	Immature	Sub-adult	44.2	10.0	N/A	NA	New	N/A
Year 3	Summer	Frog 9	GBF#	14	17/02/2021	17	South	5	4-6	Exposed leaf litter	Immature	Juvenile	39.4	6.0	N/A	NA	New	N/A

Year	Season	Frog ID	GBF	No.	Date	Zone	Creek side	Distance from stream edge (m)	Distance ranges	Microhabitat	Sex*	Age*	S/V length (mm)	Weight (g)	Breeding condition*	Microchip ID	New or recapture	Chytrid detections
Year 3	Autumn	Frog 10	GBF#	15	14/04/2021	3	North	5	4-6	Leaf litter - cover	Immature	Sub-adult	56.0	22.0	NA	911001000620123	New	N/A
Year 3	Autumn	Frog 11	GBF#	16	14/04/2021	3	North	0.4	0-2	Exposed bare earth - cover	Immature	Juvenile	39.0	NR	NR	NA	New	N/A
Year 3	Autumn	Frog 12	GBF#	17	14/04/2021	6	North	4	2-4	Exposed bare earth	Immature	Sub-adult	59.2	25.3	NA	991001000620129	New	N/A
Year 3	Autumn	Frog 13	GBF#	18	14/04/2021	10	North	8	6-8	Exposed leaf litter	Immature	Sub-adult	59.7	24.0	NA	956000010433861	New	N/A
Year 3	Autumn	Frog 14	GBF#	19	15/04/2021	8	South	2	0-2	Exposed bare earth - cover	Immature	Sub-adult	59.2	25.5	NA	956000010454091	New	N/A
Year 3	Autumn	Frog 15	GBF#	20	15/04/2021	6	South	2.5	2-4	Exposed bare earth - cover	Immature	Sub-adult	52.2	15.0	NA	956000010434396	New	N/A
Year 3	Autumn	Frog 16	GBF#	21	15/04/2021	5	South	4	2-4	Exposed bare earth - cover	Male	Adult	63.4	33.0	NA	956000010427097	New	N/A
Year 3	Autumn	Frog 17	GBF#	22	15/04/2021	5	North	NR	NR	N/A	Male	Adult	NR	NR	Calling	NR	New	N/A
Year 3	Autumn	Frog 18	GBF#	23	15/04/2021	5	South	3	2-4	Exposed leaf litter	Female	Adult	94.0	123.0	Gravid	956000010433901	New	N/A
Year 3	Autumn	Frog 19	GBF#	24	15/04/2021	4	South	3	2-4	Exposed bare earth - cover	Male	Adult	68.3	50.0	Dark	Not tagged	New	N/A
Year 3	Autumn	Frog 20	GBF#	25	15/04/2021	4	South	7	6-8	Exposed bare earth	Male	Adult	63.1	26.0	NA	Not tagged	New	N/A
Year 3	Autumn	Frog 21	GBF#	26	15/04/2021	4	South	8	6-8	Exposed leaf litter	Immature	Sub-adult	59.7	30.5	NA	Not tagged	New	N/A
Year 4	Spring	Frog 1	GBF#	23	17/11/2021	6	North	4	2-4	Leaf litter - cover	Female	Adult	98.1	122.0	Gravid	956000010433901	Recapture	Positive
Year 4	Spring	Frog 2	GBF#	3	17/11/2021	6	North	0.3	0-2	Vegetation cover	Female	Adult	87.3	88.0	NR	00077E8FEF	Recapture	N/A
Year 4	Spring	Frog 3	GBF#	25	18/11/2021	7	South	9	8-10	Exposed leaf litter	Male	Adult	66.8	36.0	Moderate	960000011419351	Recapture	Positive
Year 4	Spring	Frog 4	GBF#	26	18/11/2021	5	South	3	2-4	Exposed leaf litter	Male	Adult	63.5	42.0	Dark	960000011425829	Recapture	Possible
Year 4	Spring	Frog 5	GBF#	27	18/11/2021	5	South	6	4-6	Exposed leaf litter	Male	Adult	65.8	38.0	Dark	960000011423017	New	Positive
Year 4	Spring	Frog 6	GBF#	28	18/11/2021	4	South	0.8	0-2	Exposed leaf litter	Male	Adult	73.8	48.0	Dark	960000011408672	New	Possible
Year 4	Spring	Frog 7	GBF#	29	18/11/2021	4	South	0.1	0-2	Exposed bare earth	Male	Adult	76.1	50.0	Moderate	960000011459761	New	Possible
Year 4	Spring	Frog 8	GBF#	30	18/11/2021	7	South	7	6-8	Exposed leaf litter	Female	Adult	92.5	122.0	Gravid	960000011432455	New	Positive
Year 4	Summer	Frog 9	GBF#	31	9/02/2022	4	South	6.4	6-8	Exposed leaf litter	Immature	Juvenile	38.5	17.0	NA	NA	New	Negtative
Year 4	Summer	Frog 10	GBF#	29	9/02/2022	4	South	0.5	0-2	Exposed bare earth	Female	Adult	86.4	95.0	Gravid	960000011459761	Recapture	Negtative
Year 4	Summer	Frog 11	GBF#	32	9/02/2022	4	South	0.9	0-2	Leaf litter - cover	Immature	Sub-adult	53.9	18.0	NA	960000011425922	New	Negtative

Year	Season	Frog ID	GBF	No.	Date	Zone	Creek side	Distance from stream edge (m)	Distance ranges	Microhabitat	Sex*	Age*	S/V length (mm)	Weight (g)	Breeding condition*	Microchip ID	New or recapture	Chytrid detections
Year 4	Summer	Frog 12	GBF#	33	9/02/2022	4	South	2.3	2-4	Exposed leaf litter	Male	Adult	76.0	58.3	Dark	960000011427483	New	Negtative
Year 4	Summer	Frog 13	GBF#	34	9/02/2022	4	North	NR	NR	N/A	Male	Adult	NR	NR	Calling	NR	New	Negtative
Year 4	Summer	Frog 14	GBF#	35	9/02/2022	8	South	8	6-8	Exposed leaf litter	Female	Adult	79.5	80.0	NR	960000011431052	New	Negtative
Year 4	Summer	Frog 15	GBF#	36	3/03/2022	4	South	NR	NR	N/A	Male	Adult	NR	NR	Calling	NR	New	Negtative
Year 4	Summer	Frog 16	GBF#	37	3/03/2022	10	North	8.5	8-10	Leaf litter - cover	Immature	Sub-adult	50.3	23.5	N/A	NA	New	Possible
Year 4	Summer	Frog 17	GBF#	38	3/03/2022	11	North	3.2	2-4	Leaf litter - cover	Female	Adult	119.0	96.3	Gravid	Not tagged	New	Negtative
Year 4	Summer	Frog 18	GBF#	39	3/03/2022	11	North	4.5	4-6	Exposed bare earth - cover	Immature	Juvenile	36.6	19.0	NA	NA	New	Negtative
Year 4	Summer	Frog 19	GBF#	40	3/03/2022	13	North	0.6	0-2	Exposed bare earth - cover	Female	Adult	104.0	90.6	Gravid	Not tagged	New	Negtative
Year 4	Autumn	Frog 20	GBF#	41	11/04/2022	7	South	8.5	8-10	Exposed leaf litter	Immature	Sub-adult	52.9	22.0	N/A	960000011423778	New	Negtative
Year 4	Autumn	Frog 21	GBF#	42	11/04/2022	5	South	3.6	2-4	Exposed leaf litter	Female	Adult	91.4	130.0	Gravid	960000011432288	New	Negtative
Year 4	Autumn	Frog 22	GBF#	43	11/04/2022	7	South	6	4-6	Leaf litter - cover	Immature	Sub-adult	53.1	23.0	N/A	960000011450114	New	Negtative
Year 4	Autumn	Frog 23	GBF#	44	11/04/2022	12	North	4.5	4-6	Exposed bare earth	Immature	Sub-adult	55.2	25.0	N/A	960000011427302	New	Possible
Year 4	Autumn	Frog 24	GBF#	45	11/04/2022	13	North	9	8-10	Exposed leaf litter	Male	Adult	68.5	42.0	Moderate	960000011433481	New	Possible
Year 4	Autumn	Frog 25	GBF#	46	11/04/2022	11	North	7	6-8	Leaf litter - cover	Immature	Sub-adult	59.7	32.0	N/A	960000011421640	New	Positive
Year 5	Spring	Frog 1	GBF#	47	1/12/2022	8	South	5.0	4-6	Exposed leaf litter	Female	Adult	91.0	132.0	Gravid	956000011426414	New	N/A
Year 5	Spring	Frog 2	GBF#	48	1/12/2022	6	South	NR	NR	N/A	Male	Adult	NR	NR	Calling	NR	New	N/A
Year 5	Spring	Frog 3	GBF#	49	2/12/2022	11	North	6.0	4-6	Leaf litter - cover	Male	Adult	71.5	61.0	Moderate	956000010454481	New	N/A
Year 5	Spring	Frog 4	GBF#	50	2/12/2022	13	North	10.0	8-10	Exposed leaf litter	Male	Adult	68.4	59.0	Moderate	956000010427117	New	N/A
Year 5	Summer	Frog 5	GBF#	51	6/02/2023	13	North	3.0	2-4	Leaf litter - cover	Immature	Juvenile	33.2	8.0	N/A	NA	New	N/A
Year 5	Summer	Frog 6	GBF#	52	6/02/2023	13	North	4.5	4-6	Leaf litter - cover	Immature	Juvenile	26.9	6.0	N/A	NA	New	N/A
Year 5	Summer	Frog 7	GBF#	53	7/02/2023	5	North	NR	NR	N/A	Male	Adult	NR	NR	Calling	NR	New	N/A
Year 5	Autumn	Frog 8	GBF#	54	2/05/2023	4	South	2.1	2-4	Exposed leaf litter	Immature	Juvenile	39.7	9.0	Uk	NA	New	N/A

Appendix B

Table A1: Linear regression analysis summary output for giant barred frog records and seasonal rainfall totals for operational monitoring, WC2NH.

SUMMARY
OUTPUT

Regression Statistics	
Multiple R	0.626937929
R Square	0.393051167
Adjusted R Square	0.332356284
Standard Error	2.798149379
Observations	12

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	50.70360055	50.70360055	6.475853411	**0.029123987**
Residual	10	78.29639945	7.829639945		
Total	11	129			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	2.326329104	1.485871835	1.565632412	0.148502731	-0.98439966	5.637057868	-0.98439966	5.637057868
X Variable 1	0.005854581	0.002300633	2.544769815	0.029123987	0.000728452	0.010980711	0.000728452	0.010980711