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Received: 9 July 2025 | Revised: 18 May 2026 | Accepted: 27 May 2026

DOI: 10.1111/csp2.70338

CONTRIBUTED PAPER

Conservation Science and Practice
A journal of the Society for Conservation Biology
Open Access

WILEY

Environmental DNA detection of three threatened stream frog species suggests widespread presence of two species and widespread loss of one

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Funding information
Australian Government's National Environmental Science, Program Project 4.3

Abstract

Amphibians face severe threats globally, with the amphibian chytrid fungus *Batrachochytrium dendrobatidis* causing drastic declines in many species, resulting in small remnant populations that can be hard to find. Environmental DNA (eDNA) methods offer a promising survey tool for detecting remnant populations, given detection sensitivity and ability to screen vast, remote areas. We conducted eDNA surveys for three stream frog species: the Critically Endangered Armoured Mistfrog (*Litoria lorica*), the Endangered Waterfall Frog (*L. nannotis*) and the Endangered Australian Lacelid frog (*L. dayi*). These inhabit the Wet Tropics World Heritage Area, north-east Australia, and were heavily impacted by amphibian chytrid fungus in the 1980s and 1990s. We tested for the three species' eDNA in all catchments with historic records and adjacent catchments where they potentially occurred pre-decline, using three species-specific eDNA assays. *Litoria lorica* was only detected in samples collected 22 km downstream of the single known population. *Litoria nannotis* and *L. dayi* were detected across many catchments, including some with no records of the species post-declines. The results support on-foot survey results suggesting that *L. lorica* survived chytrid-induced declines at a single site but reveal that *L. nannotis* and *L. dayi* are more widespread than thought, showing the value of eDNA methods in detecting remnant amphibian populations post disease declines.

KEYWORDS

catchment-wide detection, chytrid disease, eDNA, Litoria, rainforest, recovery, tropics

1 | INTRODUCTION

Managing and recovering remnant populations is dependent on first finding them. For threatened stream species, including many amphibians, fish and some aquatic mammals, this is commonly done by conducting traditional surveys, such as visual and/or acoustic, on as many streams as possible in catchments where species were recorded historically, pre-decline. While traditional surveys can give important information on local presence/absence and factors such as population density and sex ratios, detection of DNA in stream water can detect

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species over entire catchments, regardless of how mountainous and inaccessible they are (e.g., Lopes et al., 2021; Sasso et al., 2017; Villacorta-Rath et al., 2021). Environmental DNA (eDNA) surveys from stream water have been successfully applied to the detection of amphibians (Lopes et al., 2021; Villacorta-Rath et al., 2021), fish (e.g., Laxmi et al., 2022; Schumer et al., 2019), reptiles (Villacorta-Rath et al., 2022), and aquatic mammal species (e.g., Jamwal et al., 2023; Webb et al., 2025).

These methods are particularly powerful for detection of stream-associated frogs, given that these species have aquatic larva and spend at least part of their adult lives in the aquatic environment. Additionally, many stream frog species can be difficult to detect via traditional methods due to weather-dependent activity and calling behavior (MacKenzie et al., 2002; Scheele & Gillespie, 2018; Sewell et al., 2010). eDNA methods can detect frog species that occur at low densities (Pilliod et al., 2013) and can detect small, remnant stream frog populations over 20 km upstream (Villacorta-Rath et al., 2021). Importantly, studies have demonstrated that eDNA analysis can detect threatened frog species thought to have disappeared (based on traditional surveys) from parts of their range (e.g., Dufresnes et al., 2020; Lopes et al., 2021; Renan et al., 2017). These detections can then target traditional surveys and conservation efforts.

Amphibians are the most threatened class of vertebrates (IUCN, 2021), with the fungal pathogen *Batrachochytrium dendrobatidis*, amphibian 'chytrid' fungus, being the most significant cause of extinction and decline in recent decades (Blaustein et al., 1994; Scheele et al., 2017; Wake & Vredenburg, 2009). Over 100 species have gone extinct globally since 1970 and hundreds more have declined, particularly from cooler parts of their ranges (Blaustein et al., 1994; Scheele et al., 2017; Wake & Vredenburg, 2009). Declines caused by amphibian chytrid fungus impacted rainforest areas in Queensland, north-east Australia, in the late 1970s and through the 1980s, and reached the Wet Tropics region in the far north-east in the early 1990s (Laurance et al., 1996; Richards et al., 1993; Scheele et al., 2017). This resulted in probable extinction of six species in the rainforest mountains of coastal Queensland and substantial declines in many others (Scheele et al., 2017). Declines were greatest in upland rainforest areas, particularly for species closely associated with streams, and remnant populations persisted in hotter parts of species' ranges where chytrid impacts were not as severe ('disease refuges'; Puschendorf et al., 2011; McKnight et al., 2017; Scheele et al., 2017).

The Armoured Mistfrog (*Litoria lorica*) was historically distributed at upland sites in the Thornton Peak uplands and Carbine Tableland above 600 m altitude in the Wet Tropics region of northeastern Australia (Cunningham, 2002; Davies & McDonald, 1979; Hoskin & Puschendorf, 2014; Puschendorf et al., 2011). *Litoria lorica* was believed to be extinct following significant declines attributed to chytridiomycosis in the early 1990s (Cunningham, 2002; Puschendorf et al., 2011). A single, small population of *L. lorica* was rediscovered in 2008 in the Carbine Tableland area, outside of its known historical range, during surveys of the Waterfall Frog (*L. nannotis*), in hotter marginal areas of the northern Wet Tropics (Hoskin & Puschendorf, 2014; Puschendorf et al., 2011). *Litoria nannotis* and another Wet Tropics stream frog, the Australian Lace-lid (*L. dayi*), also suffered extensive population declines due to chytrid disease in the late 1980s and early 1990s. These declines were particularly notable from upland areas above 600 m elevation, but both species persisted at many lowland sites (McDonald & Alford, 1999). In the decades since these declines, *L. nannotis* has recolonized some upland sites, whereas there has been poor recovery of *L. dayi* (Bell et al., 2020; McKnight et al., 2017). Since the rediscovery of *L. lorica*, traditional surveys on foot have been performed at most of the known sites pre-decline and also in many nearby catchments with extensive suitable waterfall habitat but no historic records (Hoskin & Puschendorf, 2014). These surveys failed to find other populations of *L. lorica* and found very few populations of *L. dayi*, but did find many populations of *L. nannotis* (Hoskin & Puschendorf, 2014).

Litoria lorica (Figure 1A) remains known from the single rediscovered population, and now a nearby reintroduced population upstream in the same catchment (Hoskin & Puschendorf, 2014). The species is listed as Critically Endangered under the Australian Environment Protection and Biodiversity Conservation (EPBC) Act. *Litoria dayi* (Figure 1B) is listed as Endangered on the EPBC Act. *Litoria nannotis* (Figure 1C) was formerly listed as Endangered on the EPBC Act but was recently delisted due to evidence of recovery and is listed as Endangered on the State list (Queensland Nature Conservation Act). Given their threatened status, finding populations of these species is important, particularly for *L. lorica*. However, conducting surveys on foot to locate populations of these species is challenging and time-consuming due to the remote and rugged terrain. Villacorta-Rath et al. (2021) showed that eDNA detection of *L. lorica* and *L. nannotis* is possible in water samples taken from up to 22 km downstream from known populations, providing a survey technique for entire catchments.

Here, we used an eDNA approach to test for the presence of these three threatened frog species in watersheds where they were previously known to occur in the

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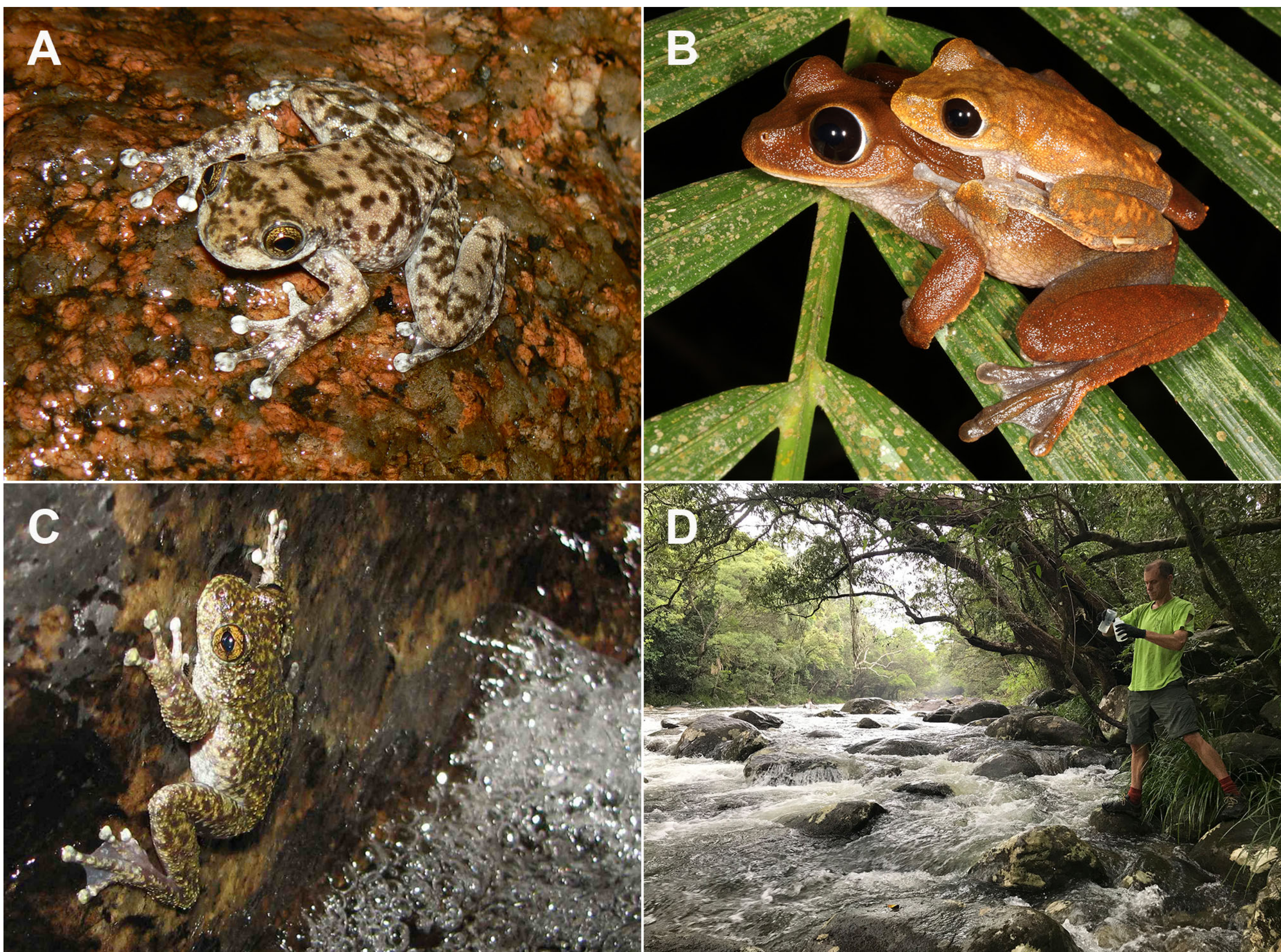

FIGURE 1 Photos of the three study species: (A) the Armoured Mistfrog (*Litoria lorica*), (B) the Australian Lace-lid (*L. dayi*), and (C) the Waterfall Frog (*L. nannotis*); and (D) water sampling at one of the study sites (Noah Creek). Photo credit: Conrad J Hoskin.

northern Wet Tropics area. We predicted that *L. dayi* and *L. nannotis* would be detected in many of the sampled catchments, but that *L. lorica* would be detected only in the catchment where the remnant population is known to occur.

2 | METHODS

2.1 | Study system

We collected water samples from 12 sites within the historical distribution of *L. lorica* (Figure 2). These sites included most major catchments draining off Thornton Peak and the Carbine Tableland, including all catchments with historical records and the single catchment with the known persistent population and the reintroduced population. These catchments also included suitable habitat for *L. dayi* and *L. nannotis*, which are of similar ecology to *L. lorica* in inhabiting streams with fast-flowing water and waterfalls. These three species fall in a species group called the 'torrent frogs'. The sampled catchments had a mix of pre- and post-decline records of *L. dayi* and *L. nannotis* (Table 1). These two species were therefore also included in the eDNA analyses to assess persistence and compare eDNA detection with records based on traditional survey methods.

2.2 | Species occurrence records pre- and post-1993

Occurrence records of the three target species were obtained from publicly available databases, such as the Atlas of Living Australia, and field surveys by one of us since 1995 (CJH). Records prior to 1993 were categorized as 'pre-decline', and those from 1993 as 'post-decline', based on the documented history of chytrid-related frog declines in the northern Wet Tropics region (Laurance et al., 1996; McDonald & Alford, 1999; Richards et al., 1993). All occurrence records come from sightings made during traditional on-foot surveys. The entire list of species occurrence sources is listed in Supporting Information S1. Water samples were collected at 12 sites

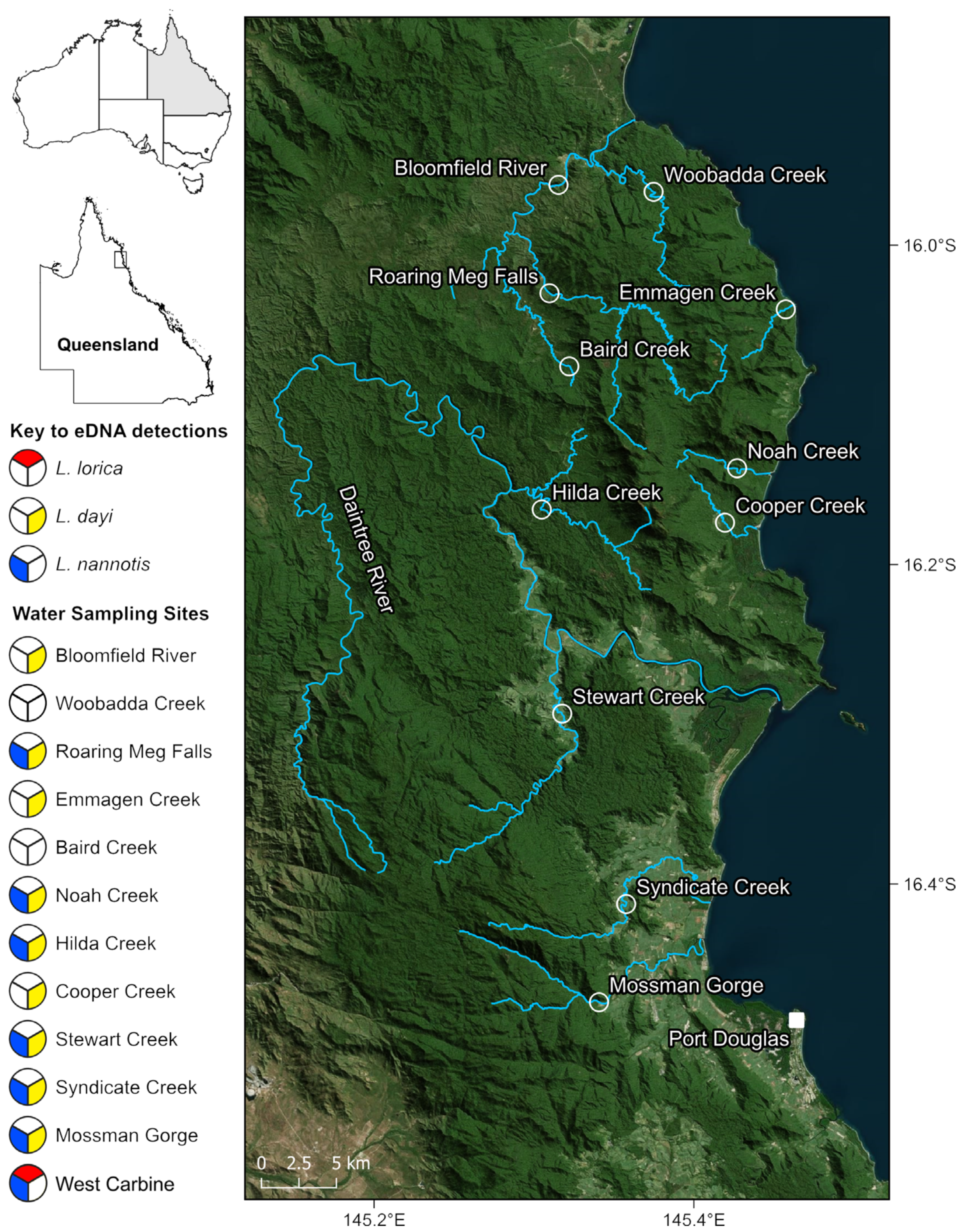

FIGURE 2 Study area in the Wet Tropics World Heritage Area, northeast Queensland, Australia. Water sampling sites are shown as open white circles on the map. The West Carbine site is not shown. A summary of the eDNA detection results is presented to the left of the map. Presence/absence of target species eDNA is shown as filled sectors in the circles: *L. lorica* (red), *L. dayi* (yellow), *L. nannotis* (blue) (see key); pale colored sectors show no detections. The top inset shows Australia. The second inset shows Queensland, with a rectangle showing the main map area.

within the Thornton Peak and Carbine Tableland areas. Eight sites were set on drainages flowing out of the Thornton Peak upland area: Bloomfield River, Woobadda Creek, Roaring Meg Falls, Emmagen Creek, Baird Creek, Noah Creek, Hilda Creek, and Cooper Creek. Four sites were set on drainages around Carbine Tableland: Stewart Creek, Syndicate Creek, Mossman Gorge, and West Carbine. (Figure 2; Table 1). Details on the water sampling locations can be found under Supporting Information S2.

2.3 | Environmental DNA sample collection

Water samples were collected between October 2019 and January 2020. For some sites, water samples were taken approximately 500 m downstream from historical or recent occurrence records, while for others, water samples were taken a considerable distance downstream from occurrence records (details on Table 1). Some water sampling sites had no upstream occurrence records but were within 22 km of potentially suitable habitat visualized on Google Earth imagery, with 22 km being the maximum downstream distance for eDNA detection of *L. lorica* and *L. nannotis* in Villacorta-Rath et al. (2021).

Five replicate 400 mL samples of water were collected at each site using a new, clean, plastic bottle for each (Figure 1D). Each of these 400 mL samples was then decanted into a plastic jar containing 150 mL of Longmire's buffer, making a total of 550 mL water sample plus preservative buffer. Additionally, a field blank was conducted at each site and consisted of decanting 400 mL of MilliQ water into a plastic jar containing 150 mL Longmire's buffer. All samples were carefully packed upright

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TABLE 1 Sampling site details, upstream occurrence records pre- and post-1993, and eDNA results for *L. lorica*, *L. nannotis* and *L. dayi*.

Location	Site name	Latitude (°)	Longitude (°)	Elevation (m)	Records pre-1993	Records post-1993	% positive eDNA field reps	% positive eDNA technical reps
(a) *Litoria lorica*								
Thornton uplands	Bloomfield River	−15.96229	145.31531	25	**Yes (29)**	No	0	0
	Woobadda Creek	−15.96661	145.37479	151	No	No	0	0
	Roaring Meg Falls	−16.03016	145.30969	202	**Yes (17)**	No	0	0
	Emmagen Creek	−16.04022	145.45747	23	No	No	0	0
	Baird Creek	−16.07592	145.32207	242	No	No	0	0
	Noah Creek	−16.13972	145.42705	29	No	No	0	0
	Hilda Creek	−16.16554	145.30484	127	**Yes (11)**	No	0	0
	Cooper Creek	−16.17377	145.41946	20	No	No	0	0
Carbine Tablelands	Stewart Creek	−16.29337	145.31764	15	No	No	0	0
	Syndicate Creek	−16.42320	145.35738	25	No	No	0	0
	Mossman Gorge	−16.47182	145.33185	90	**Yes (6)**	No	0	0
	West Carbine			370	No	**Yes (22)**	**20**	**1.7**
(b) *Litoria nannotis*								
Thornton uplands	Bloomfield River	−15.96229	145.31531	25	**Yes (18)**	**Yes (14)**	0	0
	Woobadda Creek	−15.96661	145.37479	151	No	No	0	0
	Roaring Meg Falls	−16.03016	145.30969	202	**Yes (5)**	**Yes (0.5)**	**100**	**75**
	Emmagen Creek	−16.04022	145.45747	23	No	No	0	0
	Baird Creek	−16.07592	145.32207	242	No	No	0	0
	Noah Creek	−16.13972	145.42705	29	**Yes (1.5)**	No	**60**	**21.7**
	Hilda Creek	−16.16554	145.30484	127	**Yes (9)**	**Yes (8)**	**80**	**10**
	Cooper Creek	−16.17377	145.41946	20	No	No	0	0
Carbine Tablelands	Stewart Creek	−16.29337	145.31764	15	No	No	**80**	**15**
	Syndicate Creek	−16.42320	145.35738	25	No	No	**100**	**34.5**
	Mossman Gorge	−16.47182	145.33185	90	**Yes (0.5)**	**Yes (0.5)**	**80**	**13.1**
	West Carbine			900	**Yes (31)**	**Yes (22)**	**20**	**1.7**
(c) *Litoria dayi*								
Thornton uplands	Bloomfield River	−15.96229	145.31531	25	**Yes (29)**	**Yes (15)**	**100**	**20**
	Woobadda Creek	−15.96661	145.37479	151	No	No	0	0
	Roaring Meg Falls	−16.03016	145.30969	202	**Yes (17)**	**Yes (1)**	**100**	**93.3**
	Emmagen Creek	−16.04022	145.45747	23	No	No	**20**	**1.6**
	Baird Creek	−16.07592	145.32207	242	No	No	0	0
	Noah Creek	−16.13972	145.42705	29	No	No	**100**	**31.6**

(Continues)

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TABLE 1 (Continued)

Location	Site name	Latitude (°)	Longitude (°)	Elevation (m)	Records pre-1993	Records post-1993	% positive eDNA field reps	% positive eDNA technical reps
	Hilda Creek	−16.16554	145.30484	127	No	**Yes (8)**	**40**	**13.3**
Carbine Tablelands	Cooper Creek	−16.17377	145.41946	20	No	No	**100**	**21.7**
	Stewart Creek	−16.29337	145.31764	15	No	No	**40**	**6.7**
	Syndicate Creek	−16.42320	145.35738	25	No	**Yes (0.5)**	**100**	**23.3**
	Mossman Gorge	−16.47182	145.33185	90	**Yes (0.5)**	**Yes (0.5)**	**60**	**18.3**
	West Carbine			900	**Yes (31)**	No	0	0

Note: At all sites, except West Carbine, we collected five eDNA field replicates, screened through a total of 60 qPCR technical replicates per species. At West Carbine, we collected four eDNA field replicates, screened through 48 qPCR technical replicates per species. Columns show water sampling region, water sampling site, latitude and longitude, elevation (m), yes or no for known species occurrence records pre- and/or post-1993 (with number in brackets stating the distance, in km, that the closest occurrence record was/is upstream from that water sampling site), and eDNA data. The West Carbine site is downstream from the only known extant population of *L. lorica* and specific location details are not given (to protect the frogs from threats associated with visitation). Sources of occurrence records are listed in Supporting Information S1 and qPCR detection data are recorded in Supporting Information S3, S4, and S5. All positive detections (from either traditional surveys or eDNA analysis) are highlighted in bold.

in plastic crates and returned to the laboratory. This water sampling method has previously been shown to detect *L. nannotis* and *L. lorica* at sites located up to 22 km upstream (Villacorta-Rath et al., 2021).

2.4 | Environmental DNA extractions

Immediately upon return to the laboratory (1–3 days after field sampling), 20 mL aliquots of each field replicate were decanted into five 50 mL Falcon tubes (DNA LoBind, Eppendorf). These five replicates meant that a total of 100 mL of water was screened for eDNA from each site (Villacorta-Rath et al., 2021). This water volume has been shown to give enough power to detect *L. lorica* eDNA up to 22 km downstream from a small population (Villacorta-Rath et al., 2021). Environmental DNA was extracted following a glycogen-aided precipitation method described by Edmunds and Burrows (2020). To do this, we added 20 mL isopropanol, 5 mL NaCl (5 M) and 5 μL glycogen (20 mg/mL) to each 20 mL aliquot. Samples were then incubated overnight at 4°C and subsequently spun at 6750 g for 10 min. The supernatant was then decanted and 120 μL of lysis buffer was added to eDNA pellets from each aliquot. Once pellets were dissolved in lysis buffer, pellets from all five aliquots from each field replicate were pooled into a single tube (pellet dissolved in a total of 600 μL lysis buffer) and frozen overnight at −20°C. Samples were then thawed, vortexed at maximum speed for 30 s, and incubated at 55°C for 4 h. After incubation, samples were cooled down, 1 μL glycogen and 1200 μL PEG-NaCl buffer were added, and samples were incubated at 4°C overnight. Finally, two ethanol washes were performed on each sample, and the pellet was allowed to air-dry for 10 min before adding 100 μL elution buffer. The extracted DNA was then purified using the DNeasy PowerClean Pro Cleanup Kit (Qiagen) kit and eluted in 120 μL elution buffer.

2.5 | Detection of *Litoria* species

Litoria species detection was performed using three different species-specific eDNA assays, one for each species, targeting their COI mitochondrial genes (Edmunds et al., 2019). Details of primer sequences can be found in Supporting Information S3, S4, and S5. The limit of detection (LOD) was set at 95% or greater detection (Klymus et al., 2019). Based on seven serial dilutions, the LOD for all three species was 2 copies/reaction (Supporting Information S3, S4, and S5).

All qPCR plates were set up using the EzMate™ 401 Automated Pipetting System (Arise Biotech) and run in QuantStudio5 or QuantStudio3 using 384-well plates or 96-well plates, respectively. Twelve qPCR technical replicates were run per field replicate per species to give a total of 60 eDNA technical replicates per species per site, except for West Carbine, which totalled 48 eDNA technical replicates per species due to four field replicates rather than five. In addition, each plate contained two triplicate positive control samples consisting of genomic DNA of the respective target species, as well as three no-template controls (NTC). The NTC samples did not contain the target species DNA, and their lack of amplification indicated that no contamination was introduced during plate handling.

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Each qPCR assay consisted of 3 μL of template DNA and 7 μL of master mix (5 μL PowerUp SYBR Green Master Mix; 0.5 μL forward primer at 10 μM; 0.5 μL reverse primer at 5 μM; 1 μL MilliQ® water). Thermal cycling conditions for all three *Litoria* species were as follows: initial denaturation and activation at 95°C for 2 min, then 55 cycles of 95°C for 15 s, and 60°C for 1 min. A subsequent melt curve analysis was performed to generate dissociation curves by transitioning from 60°C to 95°C, at 0.15°C s^{-1}. All plates were analyzed with a common fluorescence threshold (0.2) using QuantStudio™ Design and Analysis Software (version 1.4.2; Thermo Fisher Scientific Australia Pty Ltd) before export and subsequent analyses in Microsoft Excel.

Samples were considered putative positive detections if: (1) the amplification curve crossed the common fluorescence threshold within 50 cycles; (2) the amount of eDNA was above the LOD; and (3) the melt curve analysis showed a dissociation temperature peak at 78.52°C (±0.62%–99% confidence interval) for *L. lorica*, 79.66°C (±0.75%–99% confidence interval) for *L. nannotis*, and 79.06°C (±0.65%–99% confidence interval) for *L. dayi*. Amplicons from putative positive detections were sequenced via dual direction Sanger sequencing at the Australian Genome Research Facility (AGRF) to confirm that they were true detections. The percentage of positive detections for each species at each site was calculated based on the total number of field and qPCR technical replicates that showed positive amplification over the total number of replicates at that site.

2.6 | Relationship between eDNA detections and distance from the nearest known population

Logistic regressions were performed to test whether distance to the nearest known upstream population predicted eDNA detections of each of the *Litoria* species. For each species, two separate models were fitted: one using pre-decline upstream records and one using post-decline upstream records. In all models, distance to the nearest known population (km) was included as the independent variable, and eDNA detection was modeled as a binomial response. Only sites with positive eDNA detections for each species were included in the models.

3 | RESULTS

All positive eDNA detections for each species matched the reference sequences from GenBank at 100% similarity (*L. lorica* MZ330819 and MZ330818; *L. dayi* JN131426 and JN131438; *L. nannotis* MZ383982). *Litoria lorica* was detected at just one of the 12 sites sampled, *L. nannotis* was detected at seven out of 12 sites, and *L. dayi* was detected at nine out of 12 sites.

3.1 | Environmental DNA detections versus traditional surveys

3.1.1 | Thornton peak area

Litoria lorica eDNA was not detected at any of the sampling sites (Table 1; Figure 2), supporting the findings of traditional surveys around Thornton Peak post-1993 that suggest local extinction from this area (Cunningham, 2002; Hoskin & Puschendorf, 2014). In contrast, *L. dayi* was detected at most (six out of eight) sites in the Thornton Peak area, and *L. nannotis* was detected at three of the eight sites (Table 1; Figure 2). The Roaring Meg Creek sampling site had the highest eDNA detection rate of both species (100% field reps for both species; Table 1), which was not surprising given that both species have been detected just up to 1 km upstream from the water sampling site in traditional surveys post-1993 (Table 1). Interestingly, the Bloomfield River sampling site is approximately 14 km downstream from the Roaring Meg site, in the same broad catchment, and only *L. dayi* was detected there (in 100% of field reps; Table 1). Both *L. dayi* and *L. nannotis* have been detected approximately 8 km upstream from the Hilda Creek eDNA sampling site in traditional surveys post-1993, and indeed eDNA detections were made for both species (40% field reps for *L. dayi*; 80% for *L. nannotis*; Table 1). The only other creek with eDNA detections for both species was Noah Creek (60% field reps for *L. nannotis*; 100% for *L. dayi*). Noah creek has a pre-1993 record for *L. nannotis* but no records of either species post-1993. *Litoria dayi* DNA was detected in water samples from Emmagen Creek (20% of field reps) and Cooper Creek (100% of field reps), despite no records of the species pre- or post-1993. There were no eDNA detections for *L. dayi* or *L. nannotis* at Woobadda Creek or Baird Creek, and no occurrence records of these species pre- or post-1993 (Table 1).

3.1.2 | Carbine tableland area

Of the four catchments sampled around the Carbine Tableland, *L. lorica* was only detected in water samples from the known catchment, in the West Carbine. As for Villacorta-Rath et al. (2021), *L. lorica* was detected (in 20% of field reps) about 22 km downstream of the

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population (Table 1). There were no *L. lorica* detections in the other three catchments sampled (Table 1; Figure 2). There is a historical (pre-1993) record of *L. lorica* in the headwaters of Mossman Gorge (Cunningham, 2002) but the species was not detected in our eDNA testing there and has not been detected post-1993 by traditional surveys (Hoskin & Puschendorf, 2014). *Litoria nannotis* was detected in water samples from West Carbine, despite the sampling occurring approximately 22 km downstream of the known population (Table 1), a result that mirrors Villacorta-Rath et al. (2021) for the same water sampling site. *Litoria dayi* was not detected in West Carbine water samples and has not been detected in traditional surveys post-1993, but was detected in the headwaters pre-1993 (Table 1). Both *L. dayi* and *L. nannotis* DNA was detected at all three of the other Carbine Tableland sites: Mossman Gorge, Syndicate Ck, and Stewart Ck. Mossman Gorge had occurrence records pre- and post-1993, including contemporary presence of both species 0.5 km upstream from the eDNA sampling site (Table 1). Detection rates were high for both species at Mossman Gorge (80% of field reps for *L. nannotis*; 60% for *L. dayi*). Syndicate Creek had the highest percentage of positive *L. dayi* and *L. nannotis* eDNA detections in field replicates (100% for both species) and qPCR technical replicates (23.3% and 34.5%, respectively) (Table 1). There are no occurrence records for *L. nannotis* in this catchment and the only record for *L. dayi* is a post-1993 sighting just upstream of the water sampling site (Table 1). Stewart Creek had moderate to high rates for *L. nannotis* and low detection rates for *L. dayi*, despite no occurrence records for either species pre- or post-1993 (Table 1).

3.2 | Relationship between eDNA detections and distance from the population

The logistic regressions did not find a significant relationship between eDNA detection of *L. nannotis* or *L. dayi* and distance to the nearest known upstream populations, either using pre = or post-decline records: *L. nannotis* (pre-decline: $\beta = 4.705 \times 10^{-8}$, SE $= 4.990 \times 10^{3}$, $z = 0.00$, $p = 1.00$; post- decline: $\beta = 3.894 \times 10^{-12}$, SE $= 7.110 \times 10^{3}$, $z = 0.00$, $p = 1.00$), *L. dayi* eDNA detection (pre- decline: $\beta = -2.235 \times 10^{-8}$, SE $= 3.272 \times 10^{3}$, $z = 0.00$, $p = 1.00$; post- decline: $\beta = -4.67 \times 10^{-11}$, SE $= 6.83 \times 10^{3}$, $z = 0.00$, $p = 1.00$). Note that the relationship between *L. lorica* eDNA detection and distance to the nearest known upstream population was not tested because the species was only detected in one catchment.

4 | DISCUSSION

Our results support the conclusion from traditional surveys (Hoskin & Puschendorf, 2014; Puschendorf et al., 2011) that *L. lorica* was extirpated by amphibian chytrid fungus from most of its historical range and persists in just one catchment. In contrast, the eDNA results show that *L. dayi* and *L. nannotis* are more widespread through the region than suggested by records from traditional surveys.

As was anticipated, species were detected in almost all cases where there were contemporary upstream observations—*L. lorica* in the single known catchment, and *L. dayi* and *L. nannotis* detected at five out of five and four out of five sites, respectively, with upstream post-decline records (Table 1). Interestingly, there were also eDNA detections at many sites with no contemporary and/or historic upstream records. For example, in the Thornton Peak area, *L. dayi* was detected at three sites with no historical or contemporary records: Emmagen, Noah and Cooper Creeks, with 100% of field replicates showing positive eDNA amplification in the latter two. Also, *L. nannotis* was detected at Noah Creek, which only had occurrence records pre-chytrid declines (Table 1). Similarly, in the Carbine Tablelands area, Stewart and Syndicate Creeks had positive eDNA detections of *L. nannotis* and *L. dayi* despite only a single contemporary record of *L. dayi* just upstream of the Syndicate Creek site (Table 1). The general lack of occurrence records in our study area reflects how difficult the remote and mountainous creeks are to survey with traditional methods. In this situation, eDNA methods are an efficient tool for catchment-wide surveys for unknown populations.

The distance from the water sampling site to the closest known individuals of the three species ranged from 0.5 to 22 km (Table 1). Downstream eDNA detection distance was at least 22 km, for *L. lorica* and *L. nannotis* at the West Carbine site. This detection distance is known from detailed on-foot surveys for these species upstream from this sampling site into the catchment (Villacorta-Rath et al., 2021). Detection to at least 22 km is high compared to other eDNA surveys of stream dwelling organisms (Jo & Yamanaka, 2022). Interestingly, eDNA detection was not related to distance to nearest upstream record in this study. This is in line with another study testing the effect of water discharge and source population biomass on eDNA detections (Van Driessche et al., 2023). The authors found that the relationship between eDNA detection and distance from the source was species-dependent, with some species showing substantial variation in detection probability beyond 300 m downstream from the population (Van Driessche

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et al., 2023). In our study, all sampling sites were located well beyond 300 m from the known species occurrence record, which may have obscured a strong distance-detection relationship. Determining the effect of stream distance on detection probability would require a more specifically designed study, with more sampling sites downstream of known populations, and incorporating other factors such as adult and larval abundance, water volume and flow rate.

We only had one known false negative in our study—lack of *L. nannotis* detection at the Bloomfield site despite contemporary records 14 km upstream (Table 1). There may well have been other false negatives at sites with no occurrence records. Several methods have been proposed to reduce false negatives in eDNA studies, including increasing field replication (Furlan et al., 2016), water volume sampled (Sepulveda et al., 2019), and qPCR technical replication (Feist et al., 2018). We anticipated very low DNA copy numbers in our samples (Laporte et al., 2020; Schumer et al., 2019) due to the rarity of the frogs, large distances between the source and sampling of DNA, and large water volumes in the catchments, and hence inconsistency in detection across replicates (Ellison et al., 2006). We therefore ran a large number of technical replicates (12) (Tréguier et al., 2014; Villacorta-Rath et al., 2021). This number represented the maximum DNA extract volume that could be screened for three target species without diluting the available extracts. Another source of false negatives could be species seasonality, as observed in some amphibians (Takahara et al., 2019) and fish (Boothroyd et al., 2016; Laramie et al., 2015). In our study system, seasonal effects would be limited for adult frogs because they occupy the stream habitat year-round, but there could be seasonality in DNA input into the stream from larval stages because tadpole numbers vary seasonally (CJH, unpub. data).

Our results add to the increasing body of evidence highlighting the power of eDNA to uncover small and overlooked populations of aquatic and semi-aquatic species, including in amphibians (Lopes et al., 2021; Thomsen et al., 2016; Valentini et al., 2016). The eDNA results then pave the way for conservation. Here, *L. lorica* was detected in only one catchment, supporting the results of traditional surveys and highlighting the need for concentrated efforts to protect the last population. The eDNA detections of *L. nannotis* and *L. dayi* suggest they are more widespread than previously believed. This suggests these species survived in warmer environmental refuges from disease (Puschendorf et al., 2011) on many of the streams in this region. These results can guide conservation efforts for these threatened species. On-foot surveys in these catchments can estimate population sizes, and eDNA screening of additional catchments can further refine the current distributions of these two species. Important areas for further eDNA surveys are the southern end of the *L. dayi* distribution, in the Paluma Range, and the northern end of the *L. nannotis* distribution, north of the Bloomfield River, areas where these species are believed to have been locally extirpated by amphibian chytrid fungus (Hoskin & Hero, 2008).

One of the strengths of eDNA surveys as conducted here is that large, remote catchments can be screened from relatively accessible downstream sites. Some of the sampled sites did not have upstream species occurrence records, either pre- or post-decline, because the rugged and inaccessible terrain has limited on-foot surveys. Another strength was the use of a simple eDNA field collection method, involving directly collecting water and preserving it in a non-hazardous, non-alcohol based buffer that can keep eDNA intact for at least 3 months at tropical ambient temperature (i.e., ±28°C) (Cooper et al., 2022). This simple and fast field protocol means that non-specialists can perform sample collection and there is no need to refrigerate the samples or rush them back to the lab (Cooper et al., 2022; Villacorta-Rath et al., 2020). This method could be applied to the search for remnant populations of amphibians and other aquatic and semi-aquatic species, globally.

ACKNOWLEDGMENTS

This work was supported through funding from the Australian Government's National Environmental Science Program—Northern Australia Environmental Resources Hub, Project 4.3 (to D.B.). Thanks to Leah Carr for laboratory assistance and Scott Macor for producing the map figure. We acknowledge the Traditional Owners of the lands and waters where the field and laboratory work were conducted. We thank the Editor and two anonymous reviewers for their constructive comments, which helped to improve this manuscript. Open access publishing facilitated by James Cook University, as part of the Wiley - James Cook University agreement via the Council of Australasian University Librarians.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

qPCR detection data following the FAIR eDNA guidelines (https://github.com/FAIR-eDNA, Takahashi et al., 2025) are included in Supporting Information S2, S3, and S4.

ETHICS STATEMENT

The authors have nothing to report.

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REFERENCES

Bell, S. C., Heard, G. W., Berger, L., & Skerratt, L. F. (2020). Connectivity over a disease risk gradient enables recovery of rainforest frogs. *Ecological Applications*, *30*(7), 1–18. https://doi.org/10.1002/eap.2152

Blaustein, A. R., Grant Hokit, D., O'Hara, R. K., & Holt, R. A. (1994). Pathogenic fungus contributes to amphibian losses in the pacific northwest. *Biological Conservation*, *67*(3), 251–254. https://doi.org/10.1016/0006-3207(94)90616-5

Boothroyd, M., Mandrak, N. E., Fox, M., & Wilson, C. C. (2016). Environmental DNA (eDNA) detection and habitat occupancy of threatened spotted gar (Lepisosteus oculatus). *Aquatic Conservation: Marine and Freshwater Ecosystems*, *26*(6), 1107–1119. https://doi.org/10.1002/aqc.2617

Cooper, M. K., Villacorta-Rath, C., Burrows, D., Jerry, D. R., Carr, L., Barnett, A., Huveneers, C., & Simpfendorfer, C. A. (2022). Practical eDNA sampling methods inferred from particle size distribution and comparison of capture techniques for a critically endangered elasmobranch. *Environmental DNA*, *4*(5), 1011–1023. https://doi.org/10.1002/edn3.279

Cunningham, M. (2002). Identification and evolution of Australian Torrent Treefrogs (Anura: Hylidae: *Litoria nannotis* group). *Memoirs of the Queensland Museum*, *48*(1), 93–102.

Davies, M., & McDonald, K. R. (1979). A new species of stream-dwelling hylid frog from northern Queensland. *Transactions of the Royal Society of South Australia*, *103*, 169–176.

Dufresnes, C., Golay, J., Schuerch, J., Dejean, T., & Dubey, S. (2020). Monitoring of the last stronghold of native pool frogs (Pelophylax lessonae) in Western Europe, with implications for their conservation. *European Journal of Wildlife Research*, *66*(3), 45. https://doi.org/10.1007/s10344-020-01380-3

Edmunds, R. C., & Burrows, D. (2020). Got glycogen?: Development and multispecies validation of the novel preserve, precipitate, lyse, precipitate, purify (PPLPP) workflow for environmental DNA extraction from Longmire's preserved water samples. *Journal of Biomolecular Techniques: JBT*, *31*, jbt.20-3104-003. https://doi.org/10.7171/jbt.20-3104-003

Edmunds, R. C., Villacorta-Rath, C., Huerlimann, R., & Burrows, D. W. (2019). *Development of eDNA assays for monitoring three endangered frog species (*Litoria dayi, L. lorica *and* L. nannotis*) in Australia's wet tropics*.

Ellison, S. L. R., English, C. A., Burns, M. J., & Keer, J. T. (2006). Routes to improving the reliability of low level DNA analysis using real-time PCR. *BMC Biotechnology*, *6*, 1–11. https://doi.org/10.1186/1472-6750-6-33

Feist, S. M., Jones, R. L., Copley, J. L., Pearson, L. S., Berry, G. A., & Qualls, C. P. (2018). Development and validation of an environmental DNA method for detection of the alligator snapping turtle (Macrochelys temminckii). *Chelonian Conservation and Biology*, *17*(2), 271–279. https://doi.org/10.2744/CCB-1315.1

Furlan, E. M., Gleeson, D., Hardy, C. M., & Duncan, R. P. (2016). A framework for estimating the sensitivity of eDNA surveys. *Molecular Ecology Resources*, *16*(3), 641–654. https://doi.org/10.1111/1755-0998.12483

Hoskin, C. J., & Hero, J. M. (2008). *Rainforest frogs of the wet tropics, north-east Australia*. Griffith University.

Hoskin, C. J., & Puschendorf, R. (2014). The importance of peripheral areas for biodiversity conservation: With particular focus on endangered rainforest frogs of the Wet Tropics and Eungella. http://www.nerptropical.edu.au/sites/default/files/publications/files/NERP-TE-PROJECT-3.3-FINAL-REPORT-COMPLETE.pdf

IUCN. (2021). Table 1a: Number of species evaluated in relation to the overall number of described species, and numbers of threatened species by major groups of organisms (2021st–1st ed.).

Jamwal, P. S., Bruno, A., Galimberti, A., Magnani, D., Casiraghi, M., & Loy, A. (2023). Environmental DNA revealed high accuracy in detection of the Eurasian otter in Himalaya. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *33*(11), 1309–1320. https://doi.org/10.1002/aqc.4010

Jo, T., & Yamanaka, H. (2022). Meta-analyses of environmental DNA downstream transport and deposition in relation to hydrogeography in riverine environments. *Freshwater Biology*, *67*(8), 1333–1343. https://doi.org/10.1111/fwb.13920

Klymus, K. E., Merkes, C. M., Allison, M. J., Goldberg, C. S., Helbing, C. C., Hunter, M. E., Jackson, C. A., Lance, R. F., Mangan, A. M., Monroe, E. M., Piaggio, A. J., Stokdyk, J. P., Wilson, C. C., & Richter, C. A. (2019). Reporting the limits of detection and quantification for environmental DNA assays. *Environmental DNA*, *2*, 271–282. https://doi.org/10.1002/edn3.29

Laporte, M., Bougas, B., Côté, G., Champoux, O., Paradis, Y., Morin, J., & Bernatchez, L. (2020). Caged fish experiment and hydrodynamic bidimensional modeling highlight the importance to consider 2D dispersion in fluvial environmental DNA studies. *Environmental DNA*, *2*, 362–372. https://doi.org/10.1002/edn3.88

Laramie, M. B., Pilliod, D. S., & Goldberg, C. S. (2015). Characterizing the distribution of an endangered salmonid using environmental DNA analysis. *Biological Conservation*, *183*, 29–37. https://doi.org/10.1016/j.biocon.2014.11.025

Laurance, W. F., Mcdonald, K. R., & Speare, R. (1996). Epidemic disease and the catastrophic decline of Australian rain forest frogs. *Conservation Biology*, *10*(2), 406–413. https://doi.org/10.1046/j.1523-1739.1996.10020406.x

Laxmi, R. K., Sehgal, N., & Rawat, V. S. (2022). Environmental DNA analysis reveals the distribution pattern of a Himalayan hill stream fish. *Environmental Biology of Fishes*, *105*(11), 1575–1587. https://doi.org/10.1007/s10641-022-01350-5

Lopes, C. M., Baêta, D., Valentini, A., Lyra, M. L., Sabbag, A. F., Gasparini, J. L., Dejean, T., Haddad, C. F. B., & Zamudio, K. R. (2021). Lost and found: Frogs in a biodiversity hotspot rediscovered with environmental DNA. *Molecular Ecology*, *30*(13), 3289–3298. https://doi.org/10.1111/mec.15594

MacKenzie, D. I., Nichols, J. D., Lachman, G. B., Droege, S., Royle, A. A., & Langtimm, C. A. (2002). Estimating site occupancy rates when detection probabilities are less than one. *Ecology*, *83*(8), 2248–2255. https://doi.org/10.1890/0012-9658(2002)083[2248:ESORWD]2.0.CO;2

McDonald, K., & Alford, R. A. (1999). A review of declining frogs in northern Queensland. In A. Campbell (Ed.), *Declines and disappearances of Australian frogs* (pp. 14–22). Environment Australia.

McKnight, D. T., Alford, R. A., Hoskin, C. J., Schwarzkopf, L., Greenspan, S. E., Zenger, K. R., & Bower, D. S. (2017). Fighting an uphill battle: The recovery of frogs in Australia's wet tropics. *Ecology*, *98*(12), 3221–3223. https://doi.org/10.1002/ecy.2019

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Pilliod, D. S., Goldberg, C. S., Arkle, R. S., & Waits, L. P. (2013). Estimating occupancy and abundance of stream amphibians using environmental DNA from filtered water samples. *Canadian Journal of Fisheries and Aquatic Sciences*, *70*(8), 1123–1130. https://doi.org/10.1139/cjfas-2013-0047

Puschendorf, R., Hoskin, C. J., Cashins, S. D., Mcdonald, K., Skerratt, L. F., Vanderwal, J., & Alford, R. A. (2011). Environmental refuge from disease-driven amphibian extinction. *Conservation Biology*, *25*(5), 956–964. https://doi.org/10.1111/j.1523-1739.2011.01728.x

Renan, S., Gafny, S., Perl, R. G. B., Roll, U., Malka, Y., Vences, M., & Geffen, E. (2017). Living quarters of a living fossil—Uncovering the current distribution pattern of the rediscovered Hula painted frog (Latonia nigriventer) using environmental DNA. *Molecular Ecology*, *26*(24), 6801–6812. https://doi.org/10.1111/mec.14420

Richards, S. J., McDonald, K. R., & Alford, R. A. (1993). Declines in populations of Australia's endemic tropical rainforest frogs. *Pacific Conservation Biology*, *1*(1), 66–77.

Sasso, T., Lopes, C. M., Valentini, A., Dejean, T., Zamudio, K. R., Haddad, C. F. B., & Martins, M. (2017). Environmental DNA characterization of amphibian communities in the Brazilian Atlantic forest: Potential application for conservation of a rich and threatened fauna. *Biological Conservation*, *215*(321), 225–232. https://doi.org/10.1016/j.biocon.2017.09.015

Scheele, B. C., & Gillespie, G. R. (2018). The extent and adequacy of monitoring for Australian threatened frog species. In *Monitoring threatened species and ecological communities* (pp. 57–68). CSIRO Publishing.

Scheele, B. C., Skerratt, L. F., Grogan, L. F., Hunter, D. A., Clemann, N., McFadden, M., Newell, D., Hoskin, C. J., Gillespie, G. R., Heard, G. W., Brannelly, L., Roberts, A. A., & Berger, L. (2017). After the epidemic: Ongoing declines, stabilizations and recoveries in amphibians afflicted by chytridiomycosis. *Biological Conservation*, *206*, 37–46. https://doi.org/10.1016/j.biocon.2016.12.010

Schumer, G., Crowley, K., Maltz, E., Johnston, M., Anders, P., & Blankenship, S. (2019). Utilizing environmental DNA for fish eradication effectiveness monitoring in streams. *Biological Invasions*, *21*(11), 3415–3426. https://doi.org/10.1007/s10530-019-02056-z

Sepulveda, A. J., Schabacker, J., Smith, S., Al-Chokhachy, R., Luikart, G., & Amish, S. J. (2019). Improved detection of rare, endangered and invasive trout in using a new large-volume sampling method for eDNA capture. *Environmental DNA*, *1*, 227–237. https://doi.org/10.1002/edn3.23

Sewell, D., Beebee, T. J. C., & Griffiths, R. A. (2010). Optimising biodiversity assessments by volunteers: The application of occupancy modelling to large-scale amphibian surveys. *Biological Conservation*, *143*(9), 2102–2110. https://doi.org/10.1016/j.biocon.2010.05.019

Takahara, T., Iwai, N., Yasumiba, K., & Igawa, T. (2019). Comparison of the detection of 3 endangered frog species by eDNA and acoustic surveys across 3 seasons. *Freshwater Science*, *39*(1), 18–27. https://doi.org/10.1086/707365

Takahashi, M., Frøslev, T. G., Paupério, J., Thalinger, B., Klymus, K., Helbing, C. C., Villacorta-Rath, C., Silliman, K., Thompson, L. R., Jungbluth, S. P., Yong, S. Y., Formel, S., Jenkins, G., Laporte, M., Deagle, B., Rajbhandari, S., Jeppesen, T. S., Bissett, A., Jerde, C. … Berry, O. (2025). A metadata checklist and data formatting guidelines to make eDNA FAIR (Findable, Accessible, Interoperable, and Reusable). *Environmental DNA*, *7*(3). Portico. https://doi.org/10.1002/edn3.70100

Thomsen, P. F., Møller, P. R., Sigsgaard, E. E., Knudsen, S. W., Jørgensen, O. A., & Willerslev, E. (2016). Environmental DNA from seawater samples correlate with trawl catches of subarctic, deepwater fishes. *PLoS One*, *11*(11), e0165252.

Tréguier, A., Paillisson, J. M., Dejean, T., Valentini, A., Schlaepfer, M. A., & Roussel, J. M. (2014). Environmental DNA surveillance for invertebrate species: Advantages and technical limitations to detect invasive crayfish *Procambarus clarkii* in freshwater ponds. *Journal of Applied Ecology*, *51*(4), 871–879. https://doi.org/10.1111/1365-2664.12262

Valentini, A., Taberlet, P., Miaud, C., Civade, R., Herder, J., Thomsen, P. F., Bellemain, E., Besnard, A., Coissac, E., Boyer, F., Gaboriaud, C., Jean, P., Poulet, N., Roset, N., Copp, G. H., Geniez, P., Pont, D., Argillier, C., Baudoin, J. M., … Dejean, T. (2016). Next-generation monitoring of aquatic biodiversity using environmental DNA metabarcoding. *Molecular Ecology*, *25*(4), 929–942. https://doi.org/10.1111/mec.13428

Van Driessche, C., Everts, T., Neyrinck, S., & Brys, R. (2023). Experimental assessment of downstream environmental DNA patterns under variable fish biomass and river discharge rates. *Environmental DNA*, *5*(1), 102–116. https://doi.org/10.1002/edn3.361

Villacorta-Rath, C., Adekunle, A., Edmunds, R. C., Strugnell, J. M., Schwarzkopf, L., & Burrows, D. (2020). Can environmental DNA be used to detect first arrivals of the cane toad, Rhinella marina, into novel locations? *Environmental DNA*, *2*, 635–646. https://doi.org/10.1002/edn3.114

Villacorta-Rath, C., Hoskin, C. J., Strugnell, J. M., & Burrows, D. W. (2021). Long distance (>20 km) downstream detection of endangered stream frogs suggests an important role for eDNA in surveying for remnant amphibian populations. *PeerJ*, *9*, e12013. https://doi.org/10.7717/peerj.12013

Villacorta-Rath, C., Espinoza, T., Cockayne, B., Schaffer, J., & Burrows, D. (2022). Environmental DNA analysis confirms extant populations of the cryptic Irwin's turtle within its historical range. *BMC Ecology and Evolution*, *22*(1), 57. https://doi.org/10.1186/s12862-022-02009-6

Wake, D. B., & Vredenburg, V. T. (2009). Are we in the midst of the sixth mass extinction? A view from the world of amphibians. *In the Light of Evolution*, *2*, 27–44. https://doi.org/10.17226/12501

Webb, B., Belmer, N., & Dickson, A. (2025). Environmental variables that influence platypus (Ornithorhynchus anatinus) eDNA detection: An insight into eDNA study design for platypus occupation. *Australian Mammalogy*, *47*(2), AM24028. https://doi.org/10.1071/am24028

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How to cite this article: Villacorta-Rath, C., Hoskin, C. J., & Burrows, D. (2026). Environmental DNA detection of three threatened stream frog species suggests widespread presence of two species and widespread loss of one. *Conservation Science and Practice*, e70338. https://doi.org/10.1111/csp2.70338

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