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Mass mortality of the endangered Purple Frog, *Nasikabatrachus sahyadrensis* Biju and Bossuyt, 2003, in the Nelliyampathy Hills, Southern Western Ghats, India

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***Abstract.*—The Purple Frog (*Nasikabatrachus sahyadrensis*) is a globally endangered, ancient relic-tual frog endemic to the Western Ghats, a biodiversity hotspot of India. Here, we present observations on mass mortality of this species at the Nelliyampathy Hills, a Reserved Forest interspersed by private coffee, rubber, and tea plantations. During 25 person-hours of surveying during January to May 2024 at three sites with various land uses, including Chernalli (a rubber plantation), Nooradi (a tea plantation + village), and Kaikatty (a forest), 185 sightings of Purple frog were obtained. Direct sightings included live tadpoles (N=100; Chernalli; January) and dead tadpoles (N=75; Nooradi; March), with indirect detection of adult males based on their breeding calls emanating from underground (N=10; Kaikatty; May). Our surveys documented the persistence of a breeding population of Purple frog and recorded a mass mortality event in the adjoining plantations. Mass mortality is a cause of concern and calls for better protective measures. Histopathological studies on tadpoles that are found dead without any traumatic injury are recommended to determine the cause of the mortality and to identify measures for preventing a reoccurrence.**

Keywords. Breeding calls, dead tadpoles, EDGE species, forests, live tadpoles, rubber and tea plantations

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Introduction

A mass mortality event (MME) affecting any organism is an issue of concern locally, but such events can have potential global implications. The cases of MMEs among amphibians have been rising in recent decades due to several anthropogenic stressors and other factors, such as infectious disease outbreaks, pollutants, biotoxic agents, habitat destruction, human perturbation, ultraviolet radiation, and climate change (Blaustein et al. 1994; Dahanukar et al. 2013; Fey et al. 2015; Kwon et al. 2017). Amphibians are characterized by sensitivity to environmental conditions across multiple life stages, so they are regarded as among the most imperiled vertebrates (Stuart et al. 2004; Wake and Vredenburg 2008). More than 7% of the global amphibian taxa are on the brink of extinction and listed as Critically Endangered, and more than 40.7% of amphibian species are globally threatened (Luedtke et al. 2023; Stuart et al. 2004). Declines and MMEs of amphibians are of great concern because amphibians carry out essential roles in maintaining ecosystem health and biodiversity, serve as indicators of environmental quality, and are crucial for nutrient cycling and pest control, all of which highlight the potential for ecosystem function disruption when their populations decline or species disappear (Hocking and Babbitt 2014; Houlahan et al. 2000; Stuart et al. 2004). Although there have been no observed declines in certain amphibian species, such trends are completely undetectable or unknown for many species (Hairston and Wiley 1993; Pechmann et al. 1991). However, many, and perhaps most, amphibians are experiencing population

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declines at alarming rates and more rapidly than any other terrestrial vertebrates (Luedtke et al. 2023).

Several emerging diseases, infections, pathogens, and toxins can lead to widespread mortality and declines in frog populations (Vasudevan and Raj 2009). While many amphibian diseases are known, only a few have been documented to cause mass mortality. These include amphibian chytrid fungus (*Batrachochytrium dendrobatidis*, or *Bd*) and Ranavirus infections (Dahanukar et al. 2013; Densmore and Green 2007; Mazzoni et al. 2009; Molur et al. 2015; Skerratt et al. 2007). These infectious diseases have been responsible for approximately 4% of extinctions and 8% of endangerment among amphibians (Smith et al. 2006). But the growing threat of climate change is replacing disease as the main cause of threat status deteriorations in recent years (Luedtke et al. 2023). In addition to these factors, lethality varies significantly among amphibian species with regard to potentially toxic substances, such as the metals iron (Fe), lead (Pb), aluminum (Al), manganese (Mn), cadmium (Cd), and zinc (Zn) (Brodeur et al. 2009; Freda et al. 1990; Girotto et al. 2020; Jung and Jagoe 1995; Khangarot et al. 1985; Lefcort et al. 1998; Marcantonio et al. 2011; Rao and Madhyastha 1987; Srivastav et al. 2016).

Many factors can lead to local or global extinctions (Collins and Crump 2009; Skerratt et al. 2007). In India, approximately 42% of the endemic amphibians in the Western Ghats are threatened with extinction (Dahanukar et al. 2013; Dinesh and Radhakrishnan 2011; Dinesh et al. 2012; IUCN 2024). Population declines and MMEs often go unnoticed, but recording MMEs can help researchers to understand the vulnerability and threats to a species. Here, we report a first-ever case of mass mortality of the endangered Purple Frog *Nasikabatrachus sahyadrensis* Biju and Bossuyt 2003, from the southern Western Ghats.

The Purple Frog is an ancient relictual species endemic to the Western Ghats of India that belongs to its own family, Nasikabatrachidae (Biju and Bossuyt 2003). It is listed as Near Threatened B1ab(iii) in the IUCN Red List with an estimated extent of occurrence of 19,479.5 km^2 (IUCN 2024). Adults of Purple frog are fossorial, residing almost entirely underground throughout their lives and only emerging above ground once annually for breeding, as they are explosive seasonal breeders (Thomas et al. 2014; Zachariah et al. 2012). Breeding and oviposition occur during April and May in seasonal streams during pre-monsoon rains. Before the monsoon intensifies, the eggs hatch and the larvae disperse into the flowing stream (Thomas et al. 2014; Zachariah et al. 2012).

Rheophilous larvae of Purple frog have a dorsoventrally flattened body with tiny dorsal eyes on their head and a large keratinized oral disc, a strong tail with shallow tailfins, as well as a variety of other morphological traits that are especially suitable for life in swift-moving streams (Raj et al. 2012; Senevirathne et al. 2016). They remain submerged and are typically seen adhering to the rocky substrates of fast-flowing torrential streams by using their well-developed and specially adapted cup-like suctorial mouthparts (Dutta et al. 2004; Raj et al. 2012; Zachariah et al. 2012). They eventually grow into large tadpoles that possess strong upper and lower jaw cartilages, well-developed trabecular horns, a definable gap between the trabecular horns and the tip of the snout, and highly keratinized teeth that are useful for attachment and feeding over the rocky substrate by orienting themselves headfirst against the water current (Raj et al. 2012; Senevirathne et al. 2016; Zachariah et al. 2012). Developing tadpoles remain in the fast-flowing stream until metamorphosis (Gosner stage 26–42, about 100 days), and afterwards shift to a fossorial lifestyle (Senevirathne et al. 2016).

Materials and Methods

Study area. The present study was carried out in Nelliyampathy (10.30–10.41 N; 76.58-76.75 E), which is located in Palakkad district, Kerala, India (Fig. 1). The site is situated at the southern edge of the Palghat Gap in the Western Ghats, encompassing three forest ranges: Nelliyampathy, Alathur, and Kollangod. The overall area of the Nelliyampathy Hills is about 285 km^2 (Vijayakumar et al. 2015), with vegetation of evergreen, semi-evergreen, and moist deciduous forests as well as scattered tea, coffee, and cardamom plantations (Ramachandran and Suganthasakthivel 2010). The elevation ranges from 100 m asl in the plains to 1,700 m in the shola grasslands. This region receives southwest monsoon rains, with pre-monsoon rains in April and May followed by the onset of the monsoon by late May or early June, and lasting through October.

Survey method. The survey was carried out at three different places in the Nelliyampathy Hills region. Observations were made initially at Chernalli Rubber plantation (10.533 N; 76.661 E, 600 m asl) during the month of January 2024 and spanned an area covering 500 m^2 of a water body, totaling 10 survey hours. Species identification was carried out based on morphological congruence of the diagnostic features of the tadpoles (Raj et al. 2012; Zachariah et al. 2012) that were derived from sightings at the previously reported locality Nelliyampathy (Das 2019; Zachariah et al. 2012). Subsequently, the site at Nooradi (10.504 N; 76.674 E, 900 m asl), comprising tea and coffee plantations along with village areas, was initially visited in March 2024, and a 200 m^2 section of a stream was surveyed over a period of 6 hours. Lastly, a survey bordering a stream at the Kaikatty forest site was conducted over two days in May 2024. This third survey took 9 hours and covered an extensive area of 500 m^2. The presence of this species was deduced while it was actively calling, with vocalizations heard distinctly from underground that

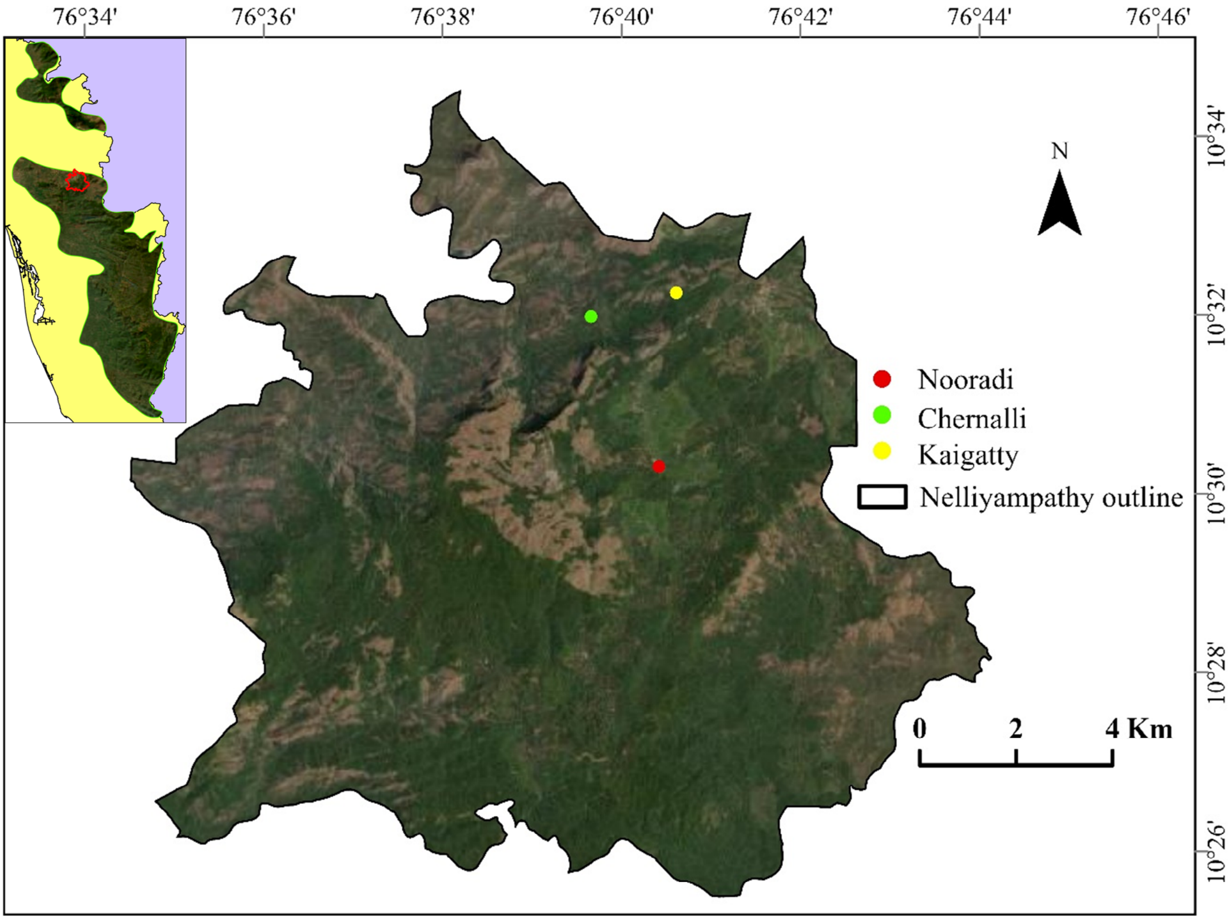

Fig. 1. Area and habitat of purple frog showing its distribution (IUCN 2020) and points of sightings (both live and dead).

accurately matched the call descriptions for this species. Call recording was carried out using a mobile phone positioned at approximately 30–50 cm from the surface of the ground where the individual was emitting its calls. We unsuccessfully attempted to get a direct sighting of an individual by removing the soil in the area from where the call was emerging.

Water sample collection. A water sample was collected to evaluate the water quality profile of the stream in Nooradi. A 500 ml sample was collected from the location where mortality was observed using a sterile sampling bottle. The sample was transported to the lab within 6 hours of collection in a cooler box, with the temperature maintained between 1 and 4 °C. The bottle was sealed tightly to prevent contamination. The sample was analyzed according to the initial parameters specified in the APHA 22nd Edition (2012). The standard values mentioned here adhere to the WHO guidelines for the quality thresholds of water deemed safe for human consumption.

Fig. 2. Live tadpoles of Purple frog in **(A)** dorsal, **(B)** lateral, and **(C)** ventral views.

Call analysis. Analyses of the call recording were performed using Audacity 3.6 software. This software was used to compute the descriptive statistics, including minima, maxima, means, standard deviations, and standard errors.

Results

The results are presented for three inter-related aspects: i. sightings of live tadpoles, ii. sightings of dead tadpoles, and iii. documentation of vocalizations, all related to the persistence of the breeding population of the Purple frog in the Nelliyampathy Hills.

Live tadpole observations (Table 1; Fig. 2). On 1 January 2024, tadpoles were sighted for the first time in the stream. The three-person survey team observed about

Table 1. Observations on Purple Frog tadpoles (both live and dead) and call surveys in Nelliyampathy, Kerala.

Site name	Habitat	Life stage	No. of sightings	Date	Area (m^2)	Survey duration (hours)	Remarks
Chernalli	Rubber / Forests	Early tadpoles	100	4 Jan 2024	500	10	Live
Nooradi	Tea / Village	Mid-stage tadpoles	50	6 Mar 2024	200	4	Dead
Nooradi	Tea / Village	Advanced tadpoles	25	8 Mar 2024	100	2	Dead
Kaikatty	Forests	Adult?	10*	20-21 May 2024	500	9	Calls

100 early-stage tadpoles in a water stream between the expansive rubber plantation and the adjoining natural forest. Despite efforts to locate adult frogs along the stream banks, none were sighted during the survey period.

Dead tadpole observations (Table 1; Figs. 3 and 4). An initial survey at Nooradi was conducted on 6 March 2024. Fifty mid-stage tadpoles were observed within a 200 m^2 area over a period of four hours. All of them were found dead without any notable signs of traumatic injuries or other marks on their bodies. The lack of traumatic injuries or visible indications of pathogen infection suggests that the environmental quality of the stream within this habitat warranted further investigation. On 8 March 2024, 25 advanced-stage tadpoles (with all the limbs fully grown) were found within a 100 m^2 area during a two-hour search in another segment of the same stream. As with the previous observation, these advanced-stage tadpoles were all dead. The water in the stream showed blackish discoloration instead of being clear and colorless.

Water quality profile (Table 2). The water sample was collected on 8 March 2024 from Nooradi. The results of the water quality analysis (Table 2) revealed deviations in several parameters from the established standard values required for the survival of aquatic life. The pH of the collected sample at 25 °C was 6.37, below the standard value of 7.4 and indicating a slight acidity. Turbidity was notably high at 14.7 mg/L, significantly surpassing the standard limit of 1 NTU and indicating the presence of suspended particles in the water. Chemical Oxygen Demand (COD) was 136 mg/L, far exceeding the recommended range of 1–10 mg/L and indicating elevated levels of organic pollution. The Total Bacterial Count exceeded 300 CFU/100 mL, surpassing the standard value of 0 CFU/100 mL and signaling high bacterial contamination of the water sample. Biological Oxygen Demand (BOD@27 ºC) was 5 mg/L, falling within the standard range but indicating the presence of organic pollutants that could potentially deplete the oxygen levels in the water. Chloride (as Cl^-) was 14.2 mg/L, well below the standard value of 200 mg/L and indicating low salinity levels. Total Hardness (as $CaCO_2$) was 26 mg/L, significantly lower than the standard value of 200 mg/L and indicating soft water quality. Total Alkalinity (as $CaCO_2$) was 30 mg/L, below the standard threshold of 200 mg/L and suggesting limited buffering capacity in the water sample. Dissolved Oxygen (DO) was less than 1.0 mg/L, falling below the standard value of 4 mg/L and indicating poor oxygen levels. Acidity was 0.7 mg/L, which was within acceptable limits. Total Dissolved Solids was measured as 55 mg/L, below the standard value of 300 mg/L and indicating low mineral content.

Fig. 3. (A) Stream passing through Nooradi township, and **(B)** stream being polluted by plastics and debris where dead tadpoles were found.

Frog vocalization observations (Table 3; Fig. 5). Calling was documented at Kaikatty (10.504 N; 76.674 E, 970 m asl), an area primarily composed of forested terrain. On 20 May 2024, in between the Ghat road and a small stream, calls were recorded between 1600 and 1700 h just after a heavy downpour. Concurrently, about

Fig. 4. Dead tadpoles of Purple frog **(A-B)** as a cluster in-situ, **(C)** dorsal, **(D)** lateral, and **(E-F)** ventral views.

Table 2. Water quality parameters of samples from polluted streams in which dead tadpoles were observed.

Sample No.	Parameter	Unit	Measured value	Standard value	Test Method from APHA 22nd Edition (2012)
1	pH at 25 ºC	-	6.37	7.4	4500 H^+ B
2	Turbidity	mg/L	14.7	1 NTU	2540 D
3	Chemical oxygen demand (COD)	mg/L	136	1 – 10	2320 B
4	Total bacterial count	mg/L	>300	0 CFU/100 mL	IS 3025-Part 39
5	Biological oxygen demand (BOD@ 27 ºC)	mg/L	5	0	IS 3025 Part 44
6	Chloride (as Cl^-)	mg/L	14.2	200	4500 Cl B
7	Total hardness (as $CaCO_2$)	mg/L	26	200	4500 SO_4^{2-}E
8	Total alkalinity (as $CaCO_2$)	mg/L	30	200	2540 C
9	Dissolved oxygen (DO)	mg/L	<1.0	4	IS 3025 Part 44
10	Acidity	mg/L	0.7	-	2320 B
11	Total dissolved solids	mg/L	55	300	2540 C

10 different individuals were heard calling at the same vicinity separated by distances of 4–6 m. Subsequently, the calls produced by the frogs subsided once the rain stopped. The call characteristics below were based on the best recorded call repertoire and depict their oscillograms/ wave forms and power spectrum plot (Fig. 5).

Call Group. The chosen segment from the recorded individual's call lasted approximately 35 sec and comprised 16 repeated call sequences. The total duration of this selected call group was 2,026.02 milliseconds (ms). Each call within this group ranged from 9.2400 to 22.9200 ms in duration, with an average duration of 13.6763 ms (Table 3).

Calls. A typical call exhibited a pulsating temporal rhythm. Calls within the call group were produced rapidly and separated by short inter-call intervals of about 16.9200 to 225.1800 ms, with an average interval of 114.5850 ms. Cumulatively, the total duration of all calls was 218.8200 ms, while inter-calls totaled 1,833.3600 ms. The amplitude of each call was characterized by its rise and fall times, and the average rise time (6.0960 ms) was shorter than the average fall time (7.5780 ms). The highest recorded amplitude within the selected call group was 0.4038 decibels (dB) (Table 3).

Pulses. The calls consisted of between 12 and 30 pulses, with an average of 20.8125 pulses. The pulse rate varied between 345 and 1,546 pulses, averaging 961 pulses per call. The first pulse and last pulse within a call had average amplitudes of 0.0920 dB and 0.1286 dB, respectively, relative to the middle pulse. Individual pulses within a call lasted between 0.0500 and 0.0662 ms on average, with an average duration of 0.0563 ms. The pulse rise time ranged from 0.01130 to 0.1088 ms, averaging about 0.0201 ms and reaching 50% of its maximum amplitude within an average of 0.0058 ms, ranging between 0.0037 and 0.0087 ms. The pulse fall time ranged between 0.0362 and 0.1625 ms and averaged about 0.0498 ms, which was more than twice the duration of the rise time. The time required for the pulse to decrease to 50% of its maximum amplitude ranged between 0.0287 and 0.0450 ms, averaging about 0.0332 ms before the end of the pulse (Table 3).

Syllable. The call syllable can be verbally described as "Trrr-tr." The initial "Trrr" denotes the long, rapid sound produced at the onset of the call, followed by "tr" which signifies a shorter sound produced immediately after the initial call, typically with a distinct gap or termination between the two sounds.

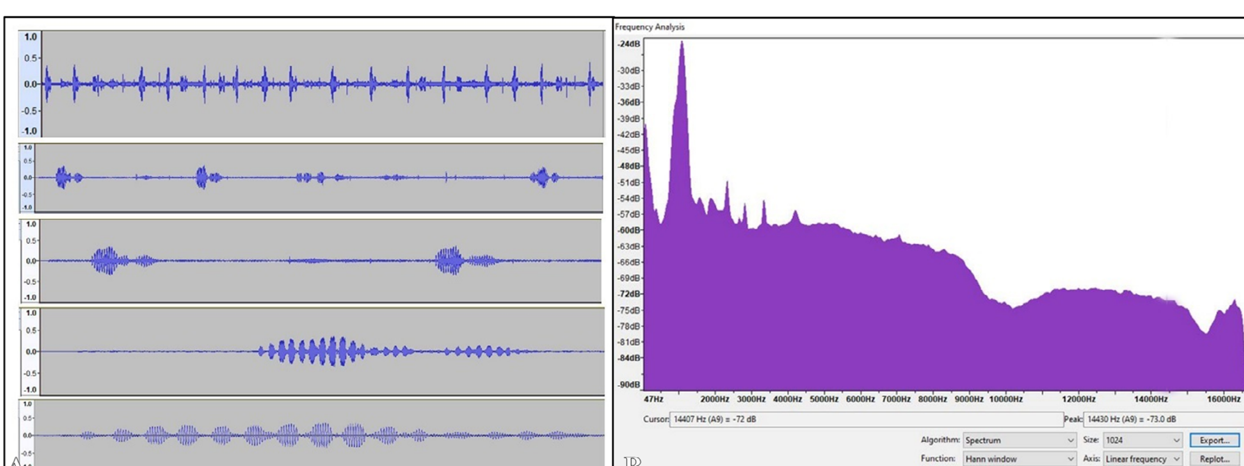

Fig. 5. Vocal repertories of Purple frog depicting **(A)** oscillograms/wave forms of a 35-s call, focusing on a series of pulsed notes, and **(B)** power spectrum plot of the same call.

Table 3. Descriptive statistics for the call group, calls, and pulses, including minimum (min), maximum (max), mean (X), standard deviation (SD) and standard error (SE), along with comparisons to the mean values of Thomas et al. (2014).

Acoustic unit	Property	Total	Min	Max	Mean	SD	SE	Thomas et al. (2014) mean values
Call group	Calls per call group	16	-	-	-	-	-	3.5
	Call group duration (ms)	2026.02	-	-	-	-	-	380.3
Calls	Pulses per call	-	12	30	20.81	458.11	114.52	6.0
	Pulse rate	-	345	1546.83	961.67	531.09	127.26	105.6
	Pulse period - first pulse (ms)	-	0.01	0.06	0.05	0.01	0.003	9.8
	Pulse period - middle pulse (ms)	-	0.05	0.06	0.05	0.004	0.001	9.3
	Pulse period - last pulse (ms)	-	0.04	0.06	0.05	0.005	0.001	9.2
	Call duration (ms)	-	9.24	22.92	13.67	4.35	1.08	59.0
	Inter call interval (ms)	-	16.92	225.18	114.58	46.55	11.63	42.5
	Call rise time (ms)	-	4.62	9.48	6.09	1.39	0.34	19.5
	Call fall time (ms)	-	3.24	14.46	7.57	3.87	0.96	39.5
	Relative peak power - first pulse (dB)	-	-0.07	0.29	0.09	6.94	1.73	-6.4
	Relative peak power - last pulse (dB)	-	0.007	0.30	0.12	6.57	1.64	-6.7
Pulses	Pulse duration (ms)	-	0.05	0.06	0.05	0.004	0.001	9.3
	Pulse rise time (ms)	-	0.01	0.10	0.02	0.02	0.005	2.7
	Pulse 50% rise time (ms)	-	0.003	0.008	0.005	0.001	0.0003	1.0
	Pulse fall time (ms)	-	0.03	0.16	0.04	0.03	0.007	6.6
	Pulse 50% fall time (ms)	-	0.02	0.04	0.03	0.003	0.001	3.9

Discussion

The observations of both live and dead Purple frog tadpoles in Nelliyampathy align closely with previously reported descriptions and depictions of the species in the literature (Raj et al. 2012; Zachariah et al. 2012). The call recordings unambiguously confirmed the species identification, corroborating earlier reports from this locality (Das 2019; Zachariah et al. 2012). As an Evolutionarily Distinct and Globally Endangered (EDGE) species, Purple frog holds significant conservation value, with an Evolutionary Distinctiveness (ED) score of 107.28 and an EDGE score of 6.76 (EDGE of Existence 2024; Gumbs et al. 2018). However, this critically important species faces a myriad of threats, underscoring the urgent need for targeted conservation efforts.

Amphibian populations worldwide are experiencing rapid and poorly understood declines (Luedtke et al. 2023; Stuart et al. 2004), and endemic species such as Purple frog, with their unique life history traits and restricted ranges, are particularly vulnerable. Between 2011 and 2020, over 1,000 breeding pairs of this species were recorded in the wild by S. Das (IUCN 2024). However, the population has been steadily declining due to habitat loss and degradation caused by urbanization, cultivation, dam construction, and other anthropogenic factors (IUCN 2024). Direct threats such as roadkill, human consumption of tadpoles, and habitat fragmentation have further exacerbated these challenges (Aggarwal 2004; Biju 2004; Das 2019; Dutta et al. 2004; Thomas and Biju 2015). Additional concerns, such as mouth abnormalities potentially linked to *Batrachochytrium dendrobatidis* (Bd) infection as reported by K. Vasudevan, raise further questions about the resilience of this species to environmental stressors, although these links remain speculative and warrant further investigation (IUCN 2024).

The current sighting of Purple frog tadpoles in Nelliyampathy, while not unexpected given previous reports of adult sightings (Das 2019; Zachariah et al. 2012), is a hopeful sign of a self-sustaining population in this region. This underscores the ecological importance of Nelliyampathy in supporting the survival of this endangered amphibian. Our findings contribute to a broader understanding of the distribution of this species and emphasize the critical role of intact natural habitats in conserving endemic wildlife.

One notable observation was the variation in call characteristics compared to those reported by Thomas et al. (2014). Factors such as the number of male vocalizations recorded, underground recording conditions, and other geo- and eco-physiographic parameters may explain these discrepancies. Intraspecific call variation has been well-documented and is known to be influenced by factors like temperature during recording (Almeida-Gomes et al. 2007; Blair 1960; Fey et al. 2015; Gerhardt and Huber 2002; Lee et al. 2016; Ryan 2001; Van Sluys et al. 2012; Velasquez 2014; Vazquez Hernandez et al. 2024). These observations warrant further research to better understand the drivers of the acoustic variation in this species.

Mass Mortality Event (MME)

Our findings suggest that the synchronous deaths of an unusually large number of Purple frog tadpoles within a restricted area and temporal window constitutes a

Mass Mortality Event (MME). Such events are often indicative of cataclysmic environmental disruptions (Gutschick and BassiriRad 2003; Haney et al. 2015; Hastings et al. 2004; Hoffmann et al. 1933; Sawyer et al. 2024; Scheffer et al. 2001; Siepielski and Benkman 2007; Till et al. 2019). The novelty of this observation further supports its classification as an MME (Anderson et al. 2017; Sawyer et al. 2024). Given the absence of external signs of predation or trauma, we suspect that anthropogenic pollution was the primary cause of this mortality. Observations of local agricultural practices revealed the use of chemicals such as urea, potash, and magnesium, as well as pesticides and herbicides, in the plantations adjacent to the affected stream. The blackish discoloration of the water and deviations in water quality parameters further support this hypothesis. Despite our inability to perform histopathological analyses due to restrictions on tissue sampling, the evidence strongly suggests that pollutants in the stream created inhospitable conditions for the tadpoles, resulting in the death of approximately 75 individuals.

The presence of garbage and other pollutants in and around the stream further compounded the environmental stressors affecting the survival of amphibian larvae. These findings are consistent with other reports linking environmental stressors to amphibian declines (Collins and Crump 2009; Lannoo 2008). As sensitive bioindicators, amphibians can highlight the deteriorating state of freshwater ecosystems, raising concerns about the broader implications for biodiversity and ecosystem services in the Western Ghats.

Conservation implications

Our findings underscore the urgent need for enhanced conservation and environmental management in the Nelliyampathy region. Although designated as a Reserved Forest, this area faces significant human pressures, including pollution, habitat fragmentation, and encroachment. Upgrading Nelliyampathy's protection status to a Wildlife Sanctuary or National Park could provide the regulatory framework needed to curb these threats and ensure the long-term survival of Purple frog and other endemic species.

In addition, immediate measures are required to monitor and mitigate pollution in streams, particularly through water quality assessments targeting heavy metals, organic chemicals, and other pollutants. Collaborative efforts involving local communities, government agencies, and conservation organizations will be essential for effectively addressing these challenges. Protecting the biodiversity of Nelliyampathy is not only crucial for species like Purple frog but also for preserving the ecological integrity and ecosystem services vital to the region's human and wildlife populations.

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