

Reproductive Phenology and Breeding Ecology of Panha's Crocodile Newt, *Tylototriton panhai*, at Phu Soi Dao National Park, Thailand

LALITA SRION¹, PANUPONG THAMMACHOTI CHARUNROCHANA^{1,2},
KANTO NISHIKAWA^{1,3,4}, WICHASE KHONSUE^{1,2} AND NOPPADON KITANA^{1,2*}

¹Program in Zoology, Department of Biology, Faculty of Science, Chulalongkorn University, 254 Phayathai Road, Bangkok 10330, THAILAND

²BioSentinel Research Group (Special Task Force for Activating Research), Department of Biology, Faculty of Science, Chulalongkorn University, 254 Phayathai Road, Bangkok 10330, THAILAND

³Graduate School of Global Environmental Studies, Kyoto University, Kyoto 606–8501, JAPAN

⁴Graduate School of Human and Environmental Studies, Kyoto University, Kyoto 606–8501, JAPAN

*Corresponding author. Noppadon Kitana (noppadon.k@chula.ac.th)

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ABSTRACT. – The Panha's crocodile newt, *Tylototriton panhai*, has a narrow distribution range in Northeastern Thailand and adjacent Laos PDR. This cryptic species is listed as vulnerable with a decreasing population trend. A local population of *T. panhai* at Phu Soi Dao National Park, Thailand with a limited distribution through the high mountainous protected area was examined for its reproductive phenology and environmental conditions inducing reproductive period. A total of 269 adult newts (257 males, 12 females) and 298 larvae were captured using a visual encounter survey method, conducted twice a day at daytime and nighttime, and four sets of three-fence arrays with pit fall traps installed at breeding sites and checked daily. The study area (breeding sites) was approximately one square kilometer at 1,633 meters above mean sea level, and was investigated from July 2020 to May 2022. Adult newts were found to be seasonal breeders, with a strongly male biased sex ratio of caught individuals, and visited breeding sites only in the wet season (May–October) with the exception of one male recorded in November 2021. Males stayed at breeding sites for several months (May–November), whereas females visited for a shorter period of time (May to July). Numbers of adults decreased in the breeding sites after breeding, whereas larvae were initially observed in June and had been found in seasonal aquatic habitats until early dry season. Both adults and larvae disappeared in March 2021. A quasi-Poisson regression model showed a significant correlation between the number of adult newts visiting breeding sites and air temperature, humidity, or water pH. Furthermore, drought-induced water scarcity influenced the duration that adults and larvae remained in aquatic habitats. Adult newts were weighed and measured for 16 morphometric characters. Females were larger in body size and exhibited larger traits than males, as shown by ANCOVA with snout–vent length as a covariate ($p < 0.05$). A total of 108 adults (99 males, 9 females) were tagged with a 7-mm PIT tag, from which 20 recaptured males (20.20%) were found from May, 2021–April, 2022. The longest duration of the same male in aquatic habitats was from May to November in 2021, but no females were recaptured in the aquatic habitats, suggesting either lower site fidelity, different habitat use, or lower survival of females during this period. The estimated population size using RMark with the POPAN model was 249 individuals with an increasing abundance trend (227–249), and the estimate detection probability for males (0.26 ± 0.04) was higher than for females (0.02 ± 0.01).

KEYWORDS: Salamandridae, crocodile newt, breeding season, environmental conditions, mark-recapture

INTRODUCTION

Newts (Amphibia:Urodela) are a group of salamanders belonging to the family Salamandridae (Frost, 2022; Raffaëlli, 2022). Adult newts spend part of their life cycle in aquatic habitats for breeding (Pough et al., 1998; Glime and Boelema, 2017; Wells, 2010). They usually have a rough and dry skin, webbed feet, and a paddle-like tail adapted for swimming in water (Duellman and Trueb, 1994; Petranks, 1998). The Salamandrid genus *Tylototriton* Anderson, 1871, well known as crocodile newts, is one group of old-world tropical newts with a restricted distribution in Asia from eastern Himalayan, Indochina, to central and southern China and southwards to northern parts of Myanmar, Thailand, Laos, and Vietnam (Nishikawa et al., 2013a, b; Sparreboom, 2014; Phimmachak et al., 2015a; Hernandez, 2016; Pomchote et al., 2021). Currently, 40 species were recognized in this genus

(Dufresnes and Hernandez, 2022), and they are classified into three species groups based on molecular phylogenetics: the *T. taliangensis*, *T. asperrimus*, and *T. verrucosus* groups (Lyu et al., 2021) corresponding to three subgenera *Liangshantriton*, *Yaotriton* and *Tylototriton*.

Although these groups were defined using genetic data, they generally align with different geographic and climatic zones. For example, species in the *T. taliangensis* group are mostly distributed in temperate montane forests of central and southwestern China, often at high elevations with cooler climates. In contrast, members of the *T. asperrimus* and *T. verrucosus* groups are more widely distributed across tropical and subtropical regions of Southeast Asia, inhabiting evergreen or mixed deciduous forests at mid to high elevations (Lyu et al., 2021; Sparreboom, 2014). These distinct environmental settings involve differences in temperature, rainfall, and the seasonal availability of aquatic habitats, all of which are likely

to influence reproductive timing and life-history strategies within the genus.

The reproductive cycles of newts vary among species due to both intrinsic life-history traits and extrinsic environmental factors (Anderson et al., 2015). For instance, *T. podichthys*, a tropical species from southern Laos, breeds in the early rainy season (June–July), with juveniles emerging in the cool dry season (December), suggesting a strong link to rainfall seasonality (Phimmachak et al., 2015b). In contrast, *T. vietnamensis* and *T. zieglerei*, found in subtropical montane habitats of northern Vietnam, breed from April to July (Böhme et al., 2005; Bernardes et al., 2017), reflecting a different climatic influence. These differences suggest that reproductive phenology in *Tylototriton* species is shaped by the interaction of intrinsic biological traits (e.g., reproductive timing, physiological thresholds) and extrinsic environmental cues (e.g., precipitation, temperature).

In Thailand, six species of the *Tylototriton* genus have been found in the Northern (*T. verrucosus*, *T. uyanoi*, *T. anguliceps*, *T. phukaensis*), Western (*T. umphangensis*), and Northeastern (*T. panhai*) parts of Thailand (Taylor, 1962; Nishikawa et al., 2013a; Le et al., 2015; Pomchote et al., 2020b, 2021). All *Tylototriton* species in Thailand have been found at high elevation at over 1,000 meters above mean sea level (AMSL) in mountainous protected areas (Taylor, 1962; Hernandez, 2016; Pomchote et al., 2020b, 2021). *Tylototriton panhai* is endemic in northeastern Thailand and western Laos (Hernandez, 2016, 2017; Hernandez et al., 2019; Phimmachak et al., 2015a). The species has only been found in protected areas and listed as vulnerable (VU) with a decreasing population trend under criteria B1 ab (iii, v) in the IUCN Red List of Threatened Species in 2020, and added in CITES Appendix II as a protected species of Thailand (Frost, 2022). Hernandez and Pomchote (2020) studied the ecology and habitat characteristics of the three main populations of *T. panhai* and provided important baseline data for this species. Furthermore, Hernandez et al. (2022) described the reproduction, courtship behavior, and egg and larval development of *T. panhai* in captivity of the phenotype II from the Phu Suan Sai-Phu Soi Dao lineage. However, basic knowledge of the life history and ecological data of local and natural populations in habitats are still unknown. Understanding the interaction between intrinsic biological traits and extrinsic environmental factors is essential to explaining how reproductive timing varies among species and across environments. This research aimed to: (1) examine the reproductive phenology of *T. panhai* at Phu Soi Dao National Park (PSDNP),

Thailand, and (2) investigate the physical factors that influence the reproductive period of *T. panhai*.

MATERIALS AND METHODS

Study site

The research was conducted during July 2020 to May 2022 at PSDNP, Uttaradit province, Thailand (Fig. 1); a part of the Luang Prabang Range that lies in a north-south direction with the eastern part of Thailand, forming a natural boundary between Thailand and Laos. The highest elevation is at 2,102 meters above mean sea level (AMSL), and the sampling area was located at approximately one square kilometer at 1,600 meters AMSL. The forest in the study area is primarily composed of *Pinus kesiya* but also includes a mixture of other native species (Hernandez and Pomchote, 2020). The area experiences an average minimum temperature of 13 °C, a maximum of 27 °C, and an average annual precipitation of 1,334.4 mm (Pongthornpruek et al., 2008). However, during the study period, temperatures at the campground ranged from 5 to 13 °C, and the precipitation varied seasonally (see below for details).

All breeding site observations and sample collections were carried out within this defined area. A total of eleven aquatic sites were surveyed to assess potential breeding habitats. These sites included ephemeral ponds, small creeks, and stream segments. Although the broader landscape consists mainly of open areas with *Pinus kesiya*, all aquatic sites were located in areas surrounded by trees and shrubs, resulting in denser vegetation cover compared to the surrounding pine-dominated forest. This denser vegetation likely contributed to increased shading and humidity at the breeding sites. Aquatic habitats varied in their physical characteristics. Most ephemeral ponds and slow-moving stream segments had muddy or leaf-litter-covered substrates, with still or gently flowing water. Water clarity ranged from clear to slightly turbid, and submerged leaf litter was commonly observed. Some sites also had noticeable algal growth. At each site, physical parameters were recorded, including site dimensions (maximum width and length), average water depth, water pH, water and air temperature, and air humidity, to assess their relationship with the occurrence of *T. panhai*.

Field survey and capturing newts

Adult newts and larvae were sought every month during the putative reproductive period (April–July), which includes the documented breeding season of May to July (Nishikawa et al., 2013; Hernandez and Pomchote, 2020; AmphibiaWeb, 2024), as well as every other month outside the reproductive period

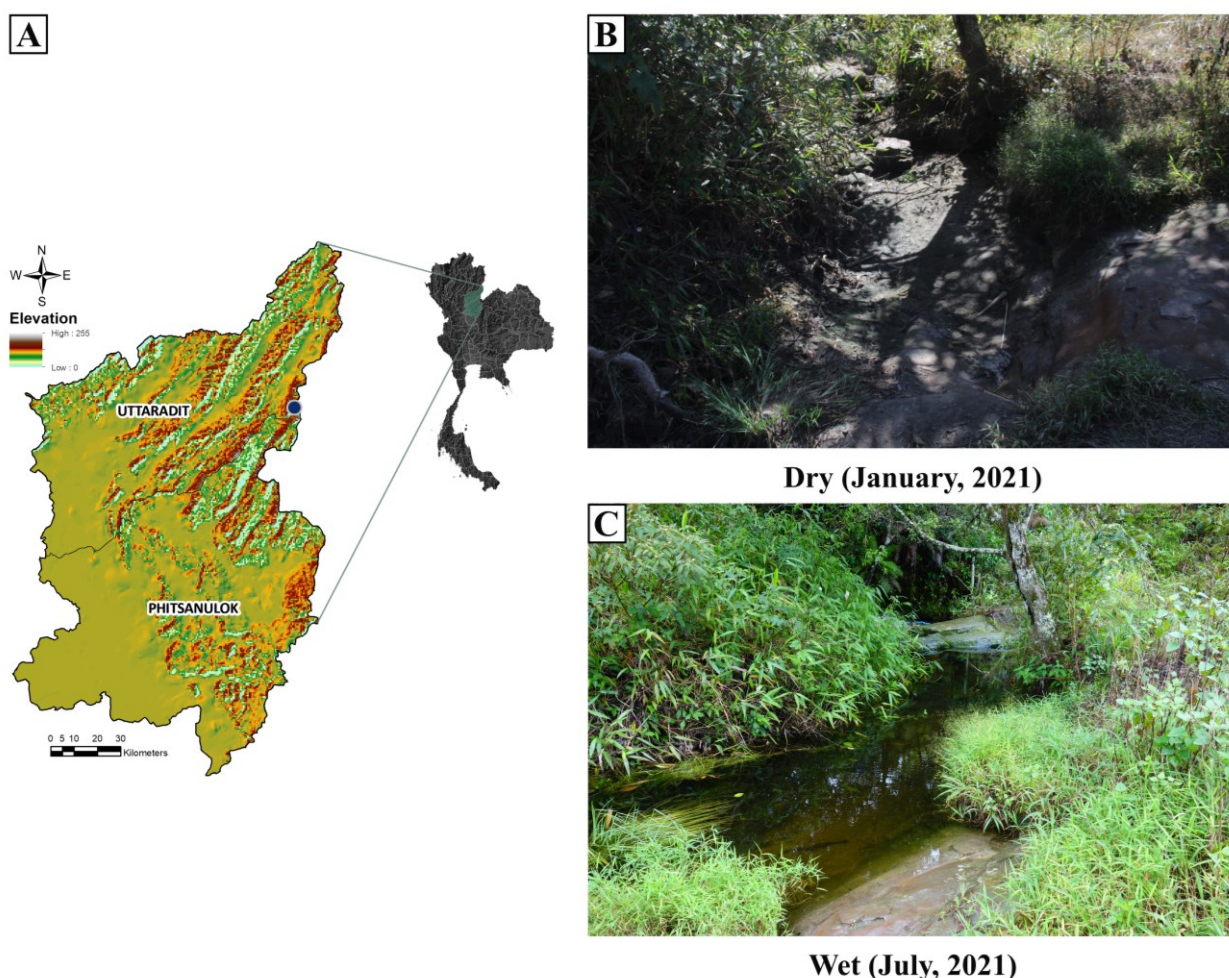


FIGURE 1. Location of study site and photos of the breeding site of *Tylototriton panhai* at PSDNP, Uttaradit province, Thailand.

(August–March). Newt surveys were conducted twice a day at daytime and nighttime (9:30–11:00 a.m.; 6:30–11:30 p.m.) according to the newts' active period (Hernandez et al., 2018). Reproductive sites (vernal ponds, creeks, and streams) were observed at the bottom of water bodies using a visual encounter survey method (Heyer et al., 1994). Adult newts and larvae were captured by hand and dip nets during the surveys, and two pairs of three-fence arrays with pitfall traps were installed at each of two locations where eggs and adults were found so as to increase the possibility of capturing adult newts. Each pair was set in the opposite direction 500 m from the breeding sites (Scott and Woodward, 1994). The three fence-arrays were comprised of three fabric fences (5 m length, 0.5 m height) with four pit-fall traps located at the center and at the terminal of each fence. A 5-L plastic bucket with a wet sponge was placed in each pit-fall and covered with 30×30 cm white corrugated plastic sheet. During each field survey, conducted over multiple consecutive days in selected months (3–5 days per month), traps were checked daily, and captured newts were kept in labeled plastic bags before being brought back to the campground. In addition, the physical characteristics of

the reproductive sites (e.g., pond width, length, and water depth), along with abiotic environmental variables such as air and water temperatures, relative humidity, and water pH, were recorded directly in the field during each observation to investigate suitable environmental conditions for breeding in *T. panhai*. At each site visit, water depth was measured at three times randomly within the site, and the average of these three measurements was used as the representative water depth for analysis. In addition to field-collected environmental variables, monthly weather data (temperature and rainfall) for Uttaradit province from January 2020 to December 2022 were obtained from the Thai Meteorological Department (TMD) to assess long-term seasonal patterns. These data were used to classify the regional climate type and determine the timing of wet and dry seasons. The Köppen-Geiger climate classification system was applied to categorize the study area as tropical wet and dry ("Aw"), and a climograph was constructed following the method of Bagnouls and Gaussen (1954) to distinguish seasonal boundaries based on the intersection of temperature and precipitation curves.

Data collection

A total of 269 captured Panha' crocodile newts were sexed based on secondary sexual characteristics, including a swollen and wider cloacal opening in males, compared to a flatter and narrower cloaca in females. After sex determination, individuals were anesthetized with 0.1% tricaine methanesulfonate (MS222) for short-term handling and morphometric measurements. All individuals recovered fully from anesthesia, with 100% survival throughout the study. Adult newts were weighed (wet weight) with an electronic balance (TANITA 1579) to the nearest 0.01 g. Sexual size dimorphism (SSD) was investigated via 16 morphometric characters, each measured using a vernier caliper to the nearest 0.05 mm following Segle et al. (2016). These morphometric characters were the total length (TTL), snout to vent length (SVL), head width (HW), head length (HDL), internarial distance (IND), eye-naris distance (END), eye length (EL), humerus length (HML), radius length (RDL), femur length (FML), tibia length (TBL), cloacal width (CLW), cloacal length (CLL), cloacal height (CLH), tail length (TL), and tail height (TLH). All adult newts were photographed from dorsal, ventral, and lateral views to document coloration and morphology. For individual identification, 108 adults were tagged with a 7-mm PIT tag (FAREAD[®]), inserted subcutaneously on the right side of the mid-body area. Antibiotic cream was applied to prevent wound and infection. Newts were kept in captivity for 24 hours before being released back to the same natural habitat (Welsh-Appleby, 2015; Whiteman et al., 2016). Detection of recaptured newts was detected using FRD5910 Animal Handheld Reader, FAREAD[®]. The field study of mark-recapture had five sampling occasions. The samplings were conducted once per month between May and July 2021, November 2021, and May 2022. The experimental protocol was approved by the Animal Care and Use Committee of Faculty of Science, Chulalongkorn University (Protocol Review No. 2123003) and the research project was also approved by The National Parks, Wildlife and Plant Conservation Department (DNP) under permit number 6410602.

Data analysis

Reproductive phenology of *Tylototriton panhai* was verified from the total number of adults captured in reproductive sites during the study period (Supplementary material S1). Correlation between counts and environmental factors of interest (air temperature, humidity, rainfall, water depth, and water pH) were analyzed using quasi-Poisson regression with the GLM function. However, because air temperature had a strong correlation with water temperature ($r =$

0.95) and water depth ($r = -0.82$), water temperature, and water depth were removed from the model (Supplementary material S2). Weight and size were compared between males and females. Weight was analyzed using a Wilcoxon rank-sum test, and sexual size dimorphism (SSD) in morphometric traits was assessed using Analysis of Covariance (ANCOVA) with snout-vent length (SVL) as a covariate, allowing us to test whether body proportions differed between sexes independent of overall body size (Supplementary material S3). For the mark-recapture analysis, mark-recapture data was analyzed from the recapture rate using the Jolly-Seber open-mark recapture models and the POPAN formulation (Schwarz and Arnason, 1996). Apparent survival (Φ , ϕ), capture probability (p), and population size (N_{super}) were analyzed using marked and RMark packages. The best model from lowest ΔAIC and the most weight $\{\Phi(\sim\text{time})p(\sim 1)\text{pent}(\sim\text{time})N(\sim\text{sex})\}$ was used to estimate apparent survival, capture probability, and population size (Supplementary material S4). All analyses were performed using R program version 4.3.0. (R Core Team, 2022).

RESULTS

Season clarification

The Köppen-Geiger climate classification categorized the study area as a tropical wet and dry climate "AW", as the annual temperature data are higher than 18 °C, and the driest month showed a precipitation of less than 60 mm and below average precipitation (Köppen, 1900; Geiger, 1961; Kottek et al., 2006). According to Bagnouls and Gaussen (1954), the climatic diagram (climograph) at the study area was plotted to determine the wet and dry months, and the wet season was verified when the temperature curve crossed over the precipitation line (Fig. 2). On average, from January 2020 to December 2022, the wet season began in April and ended in October, while the dry season began in November and ended in March. The average monthly temperature was 29.4 °C (range 24.8–33.4 °C), while the average monthly precipitation was 114.1 mm (range 0–787 mm). The mean monthly precipitation varied across the 3-y period, with a maximum precipitation of 787 mm in August 2020. However, rainfall in August 2021 and 2022 decreased dramatically to 186.2 mm and 254.8 mm, respectively.

Reproductive phenology

A total of 269 individuals (257 males, 12 females) were captured with a male-biased sex ratio (Fig. 3). All the adult newts were captured during the wet seasons except for one adult male in November 2021. Adult newts likely visited breeding sites were found from the beginning of the wet season to the early dry season

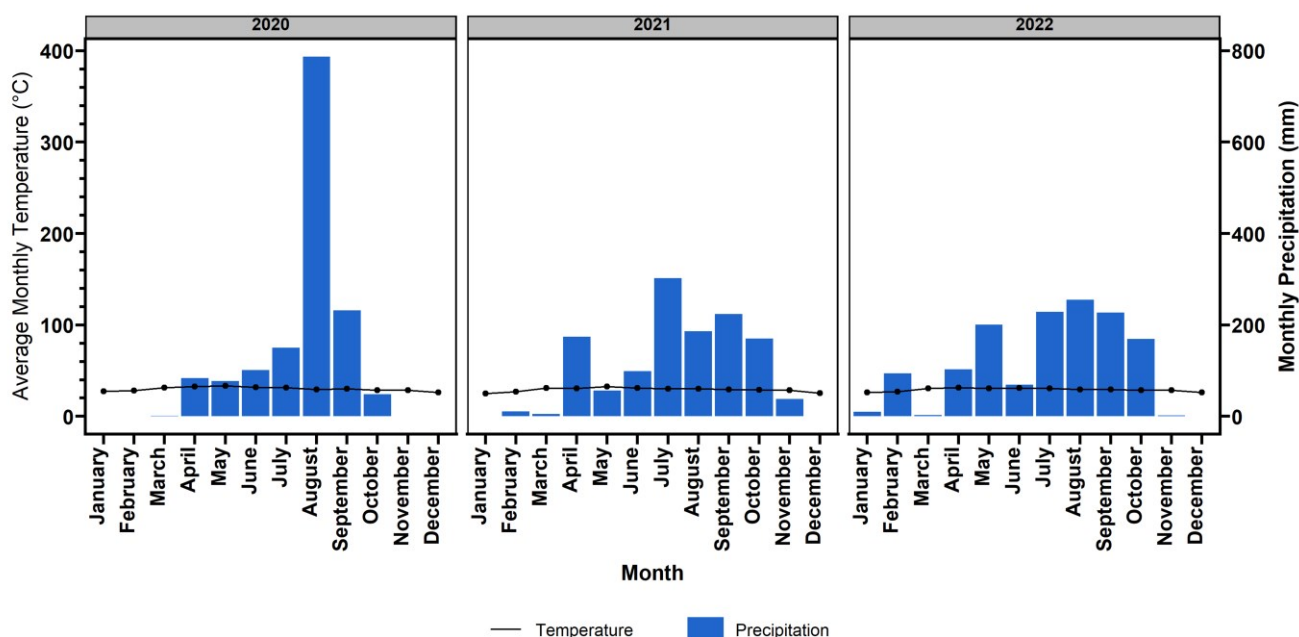


FIGURE 2. Climograph showing the average monthly temperature (°C) and monthly precipitation (mm) in Uttaradit province, Thailand (January 2020 – December 2022), with the wet season defined as the period during which the average monthly rainfall exceeded twice the average monthly temperature. Data were taken from the TMD.

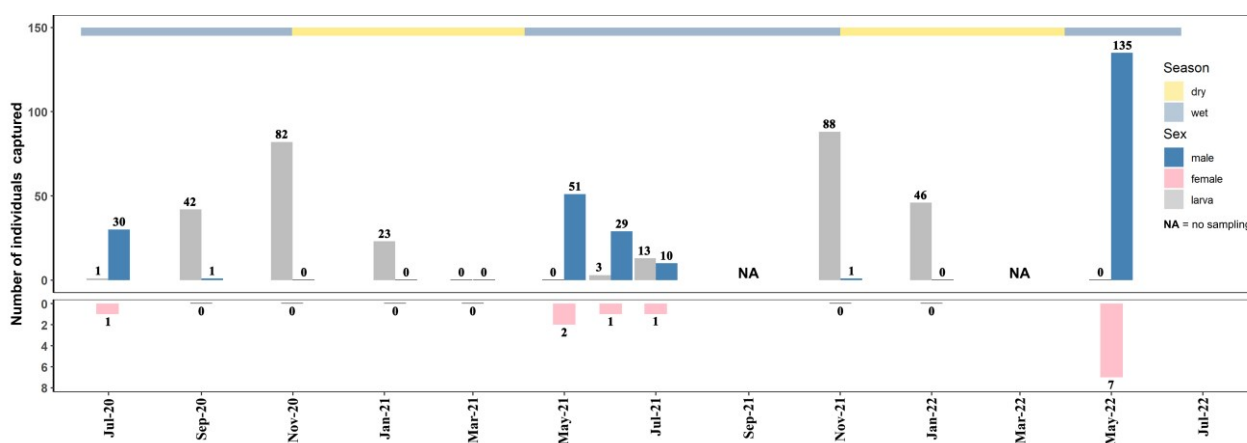


FIGURE 3. The numbers of adults and larvae of Panha's newt (*T. panhai*) recorded at PSDNP (July, 2020–May, 2022). Values represent pooled captures from visual census and traps. Months with no survey effort are indicated as NA.

(May–November). The highest numbers of males and females caught peaked in May 2022 (135 males, 7 females) and May 2021 (51 males, 2 females), respectively. The number of males captured decreased markedly in June, and the last male was caught in November 2021. Male newts individually identified using PIT tags were recaptured at the breeding sites over several months (May–November 2021), suggesting a prolonged duration of presence at the breeding sites. Male newts stayed at the breeding sites for several months (May–November 2021), while females visited reproductive sites only in a few months in the early wet season about 64 days (11 May–14 July 2021). There were no females captured from

September 2020 to March 2021, and no females were found in November 2021 or January 2022. Adult newts were not observed in the water from the late wet season to the dry period (September–March); however, we were unable to detect where they emigrated to from the water habitats after breeding. Larvae were spotted in the water in June 2021 and July 2020 after the adults had immigrated to breeding sites, with the maximum number of larvae recorded in the late wet season in November 2020 and 2021. The number of larvae captured in the following months was decreased drastically throughout the dry season, eventually dropping to zero between March 2020 and May 2021.

TABLE 1. Results of the quasi-Poisson regression analysis for the count of environmental factors, where significance levels are shown as *, **, and *** for $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

	Estimated	Std. Error	<i>t</i> value	<i>p</i> values
(Intercept)	-126.300	34.990	-3.611	0.01122 *
Air temperature	2.961	0.693	4.272	0.00525 **
Humidity	1.069	0.271	3.940	0.00762 **
Rainfall	0.000	0.002	0.179	0.86388
Water pH	-5.014	1.095	-4.580	0.00377 **

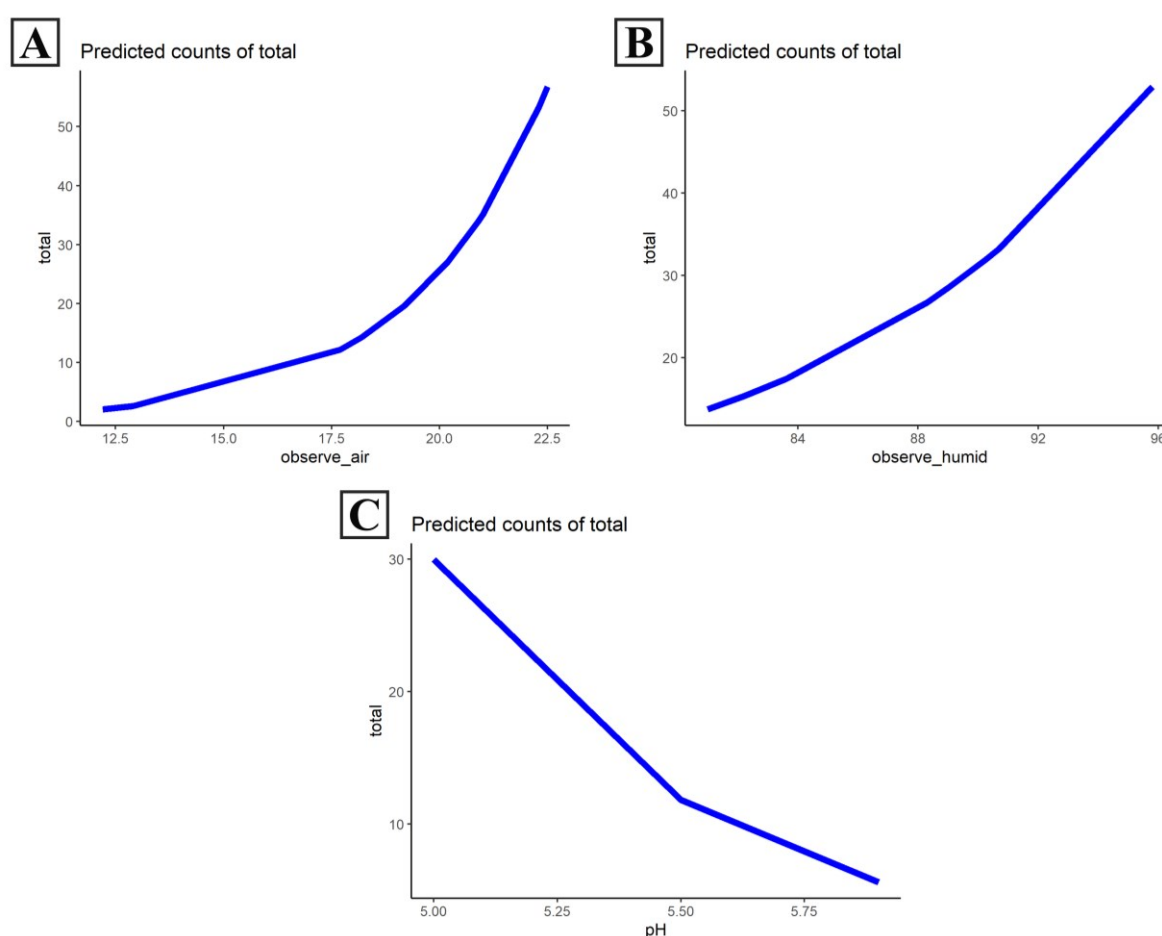


FIGURE 4. Results of GLM with a quasi-Poisson model estimates relating the number of adult newts caught to the (A) air temperature, (B) humidity, and (C) water pH.

Breeding ecology

Environmental conditions influenced adult newt appearances at breeding sites. A quasi-Poisson regression model showed that the air temperature, humidity, and water pH had significant correlations with the number of adult newts visiting breeding sites (Table 1).

The air temperature at the breeding sites ranged between 6 °C and 28.5 °C. The humidity ranged from 51 to 100, and the water pH ranged between 5 and 6. Increasing air temperature and humidity showed a significantly positive correlation with an increased number of adult newts being caught, while the water pH showed a negative correlation with the number of adult newts caught at breeding sites (Fig. 4). For

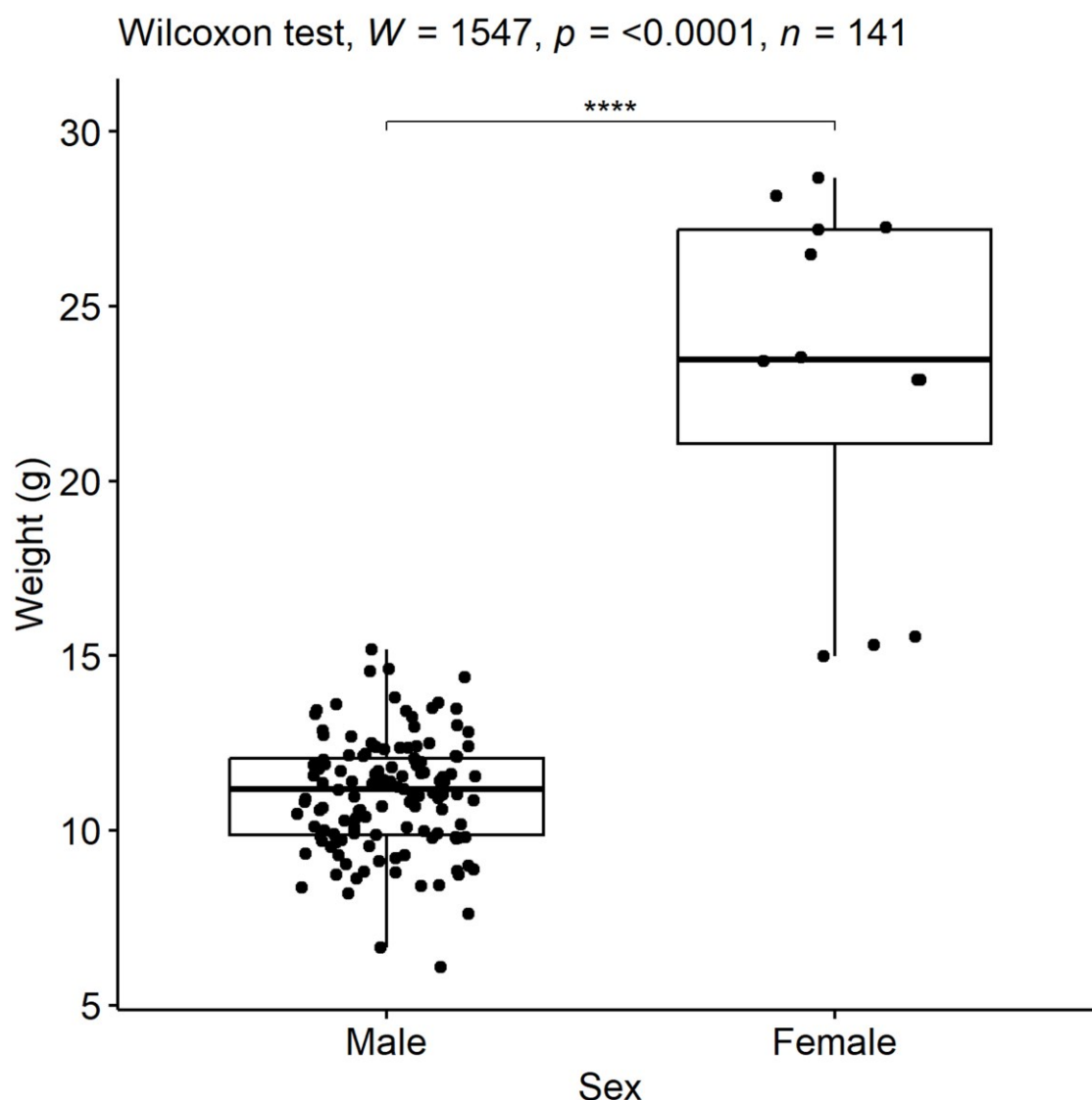


FIGURE 5. Box plot of the Wilcoxon rank-sum test for weight comparison between male and female *T. panhai*.

covariate factors with air temperature, the average monthly humidity ranged from 81% in January 2021 and 81.4% in January 2022 (dry season) to 95.8% in July 2021 (wet season). Water availability at the breeding sites fluctuated with an average maximum water depth at breeding sites of 73 cm in March 2021 and decreasing in the following months to the lowest water depth of 11.5 cm in January 2022 and 11.7 cm in January 2021.

Body condition and sexual size dimorphism in adults of *T. panhai*

There was a noticeable difference in the body weight and size between male and female *T. panhai* (129 males, 12 females). A Wilcoxon rank-sum test indicated that females were significantly heavier than males ($W = 1547$, $p < 0.0001$) (Fig. 5). To assess

sexual size and shape dimorphism in morphometric traits, we performed ANCOVA with snout–vent length (SVL) as a covariate and sex as a fixed factor. The analysis revealed that 14 out of 15 traits (excluding SVL) differed significantly between sexes after controlling for overall body size, with females showing proportionally larger values than males in most measurements ($p < 0.05$; Table 2). The only exception was cloacal height (CLH), which did not differ significantly between sexes ($p = 0.723$). These results indicate that sexual size dimorphism in *T. panhai* is not solely due to differences in overall body length, but also in body shape, with females exhibiting larger proportional dimensions across most morphological traits.

TABLE 2. Descriptive statistics of body weight (g) and morphometric measurements (mm) of male and female Panha's newts (*T. panhai*). Body weight was compared between sexes using a Wilcoxon rank-sum test, and morphometric variables were compared using ANCOVA with snout-vent length (SVL) as the covariate. Levels of statistical significance are shown as: */▲ for $p < 0.05$, **/▲▲ for $p < 0.01$, ***/▲▲▲ for $p < 0.001$, */▲▲▲▲ for $p < 0.0001$, and N.S. for $p > 0.05$. Triangle (▲) indicates Wilcoxon test, and asterisk (*) indicates ANCOVA.

Variables	Male (n = 130)			Female (n = 12)			W-value	
	Mean ± SD	Min	Max	Mean ± SD	Min	Max	/ANCOVA p (Sex)	p-value
1. Weight	11.03 ± 1.63	6.08	15.18	23.02 ± 5.10	14.99	28.67	1547.0	▲▲▲▲
2. SVL	73.99 ± 3.62	62.20	83.00	91.18 ± 5.90	82.70	99.60	-	-
3. TTL	143.01 ± 8.28	116.90	167.20	169.95 ± 10.82	153.80	183.20	0.0000	****
4. HW	15.62 ± 0.76	13.30	17.90	18.43 ± 0.90	17.20	19.70	0.0000	****
5. HDL	15.46 ± 2.62	12.15	26.50	18.70 ± 2.35	15.80	24.60	0.0001	***
6. IND	5.82 ± 0.54	4.40	7.10	6.47 ± 0.41	5.30	6.90	0.0001	***
7. END	4.64 ± 0.46	3.65	6.85	4.97 ± 0.34	4.20	5.40	0.0144	*
8. EL	4.51 ± 0.46	3.10	5.60	4.81 ± 0.43	4.30	5.50	0.0246	*
9. HML	10.97 ± 1.44	7.23	15.50	11.90 ± 1.52	8.76	13.70	0.0250	*
10. RDL	17.73 ± 1.07	14.80	20.80	19.82 ± 1.43	17.20	21.80	0.0000	****
11. FML	9.88 ± 1.29	6.97	15.80	12.18 ± 1.63	10.00	15.40	0.0000	****
12. TBL	18.83 ± 1.32	12.40	22.60	21.56 ± 1.28	19.20	23.50	0.0000	****
13. CLW	5.84 ± 0.84	4.00	8.06	3.96 ± 0.44	3.20	4.70	0.0000	****
14. CLL	6.64 ± 0.86	4.30	8.77	3.48 ± 0.78	2.30	4.80	0.0000	****
15. CLH	1.83 ± 0.55	1.00	2.63	1.90 ± 1.00	0.00	3.50	0.7228	N.S.
16. TL	70.24 ± 5.39	57.00	84.70	79.08 ± 6.09	70.20	90.98	0.0000	****
17. TLH	9.49 ± 0.99	5.52	12.61	10.24 ± 1.21	8.10	11.80	0.0094	**

Capture-recapture of adult newts

A total of 108 tagged adult newts (9 females, 99 males) were observed at the breeding sites. The lowest ΔAIC showed a constant apparent survival (Φ), probability of capturing (p) was time dependent, while the entry probability (p_{end}) and population size (N_{super}) were sex dependent. The Φ value for the top POPAN model was 0.69 (0.61, 0.76), while the RMark estimated N_{super} of *T. panhai* at PSDNP was 249.3351 with an increasing abundance from about 227 to about

249 individuals from May, 2021 to April, 2022 (Fig. 6). There was no evidence of any of the captured females returning back to the breeding sites during the observation period (2020-2022). However, 20 male newts were recaptured (20.20%) during this period, with a higher estimated detection probability for males (0.26 ± 0.04) than females (0.02 ± 0.01). The longest period the same male was detected at the breeding sites was from May to November in 2021, and he was found again in April, 2022 (Supplementary material S5).

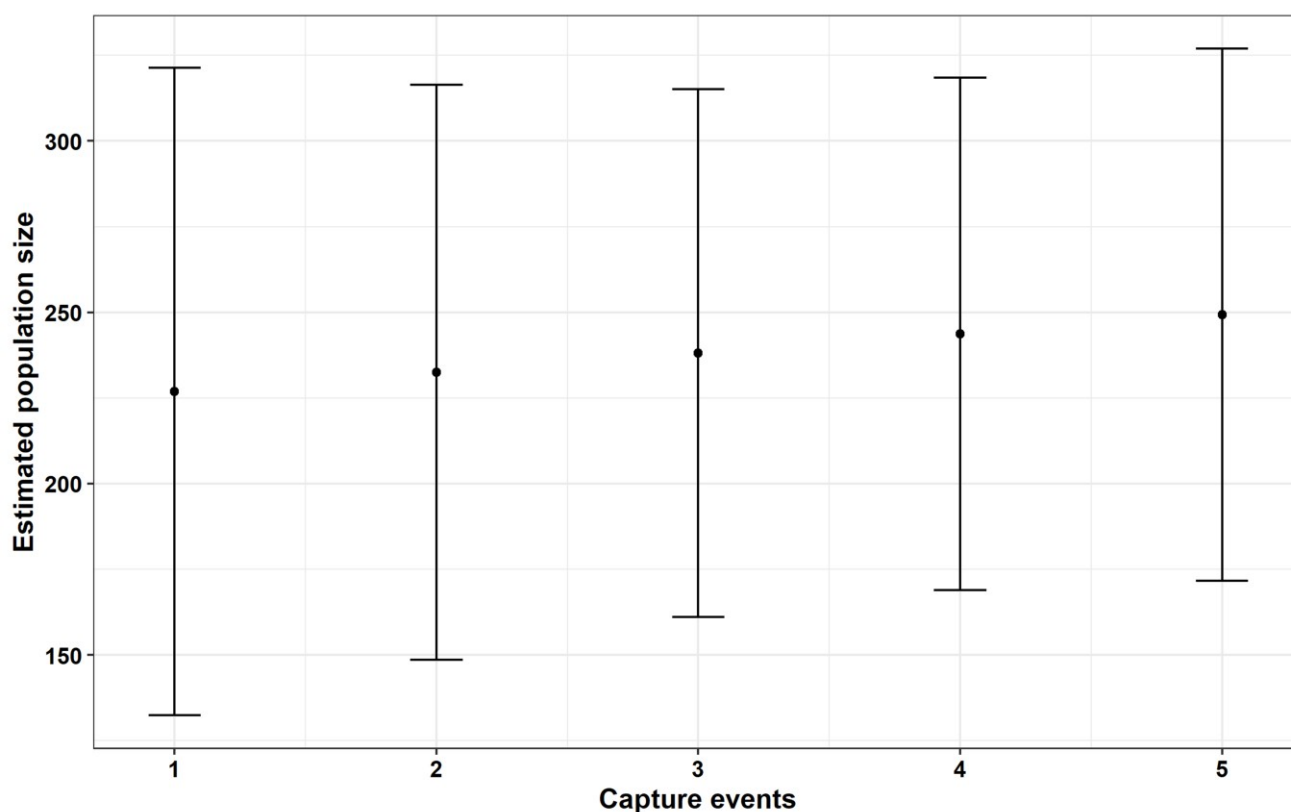


FIGURE 6. Estimated population size with error bars (95% confidence intervals, CI) of *T. panhai* at PSDNP, Thailand based on the Jolly-Seber model of five capture events from May 2021 to April 2022.

DISCUSSIONS

Breeding phenology

Adult Panha's crocodile newts (*T. panhai*) at PSDNP, Uttaradit province, Thailand were confirmed to be seasonal breeders. Hernandez and Pomchote (2020) reported the breeding period of *T. panhai* in Thailand ranged from May to July in different study locations (Phu Hin Rong Kla National Park, Phu Luang Wildlife Sanctuary, Phu Suan Sai National park), and only few individuals were found on land in August at Phu Hin Rong Kla National Park. Results of our study are compatible with previous studies including Hernandez (2016), Hernandez et al. (2019) and Hernandez and Pomchote (2020) with an extended active time for adult newts staying in water habitats, where male newts spent more time in the water during the wet season (May–November). In contrast, females only spent a few months at breeding sites during the early wet season (May–July). This breeding period pattern resembles those seen in the closely related species. The breeding season was from June to July for *T. podichthys* in Xiengkhouang province, Laos (Phimmachak et al., 2015b), between April and July

for *T. vietnamensis*, and *T. zieglerei* in northern Vietnam (Bernardes et al., 2017), between March and May, and occasionally until October for *T. himalayanus* from western Bengal, India (Kuzmin et al., 1994), and from April to July for *T. sini* from Guangdong, southeast China (Lyu et al., 2021).

According to our research, female newts not only had a larger overall body size but also showed proportionally larger morphometric traits than males after controlling for body length (SVL). They were caught in markedly lower numbers at the breeding sites than males. Our observation for the larger body size in female is similar to that for the congener species *T. himalayanus* from the Himalayan region (Seglie et al., 2010), *T. himalayanus* from western Bengal (Bedi et al., 2021), and *T. podichthys* from Laos (Phimmachak et al., 2015a), although the sex ratio in *T. podichthys* was not significantly different from the expected 1:1 ratio. This pattern in SSD is common in amphibians (Halliday and Verrel, 1986; Shine, 1978; Halliday and Tejedo, 1995). Shine (1979) reviewed the published literatures of many amphibian species and reported larger females in most anurans and 61% of urodeles. The larger body size in females is correlated with an increased fecundity, in terms of a larger egg size and

clutch size (Hedrick and Temeles, 1989; Wells, 2010). The putative shorter period of time staying at breeding sites in female may reflect that left the site immediately after laying eggs (Pimentel, 1960). However, males remained at the breeding site longer, as they may obtain energy from feeding on food in the water habitats to continue spermatophore production and courtship activities (Griffiths, 1986).

Environmental conditions on *T. panhai* breeding phenology

Based on this 2-year survey, the orientation of adults and larvae at breeding sites was related to environmental parameters, particularly air temperature, humidity, and the presence of standing water. In the early wet season, adult newts were observed immigrating to breeding sites during periods of rapidly increasing air temperature and humidity, and they occupied aquatic habitats that were already filled with water. Subsequently, adult newts emigrated and returned to terrestrial habitats, whereas a few larvae were detected in the water in June 2021. The orientation between adults and larval stages at breeding sites may avoid competition between the different stages (Griffiths, 1986; Smith, 1983). Larvae remained in the water after adults had dispersed, and the highest numbers of larvae were recorded in November during the late wet season in both years. After November, larval presence gradually declined throughout the dry season and eventually dropped to zero between March and May of the following year. An absence of all stages of newts at the breeding sites was detected in the late dry season (March-May) as the water level was quickly reduced and had dried up in most of the breeding sites by the late dry season; however, other breeding sites consisted of shallow water (Fig. 1B). The response to water availability may differ between different aquatic organisms based on their life history strategy and adaptability, while the timing of metamorphosis may be correlated with the drying of water habitats (Semlitsch and Wilbur, 1988; Anderson et al., 2015, Wilcox et al., 2017). To this end, *T. panhai* larvae may circumvent the limited water and food availability at breeding sites in the late dry season by completing larval growth and metamorphosis before all the water at the breeding sites has evaporated.

Circumneutral water pH at breeding sites is commonly preferred in *Tylototriton* species. For example, the pH of the breeding pond water was found to be between 6.8 and 8 for *T. zieglerei* (Bernardes et al., 2017), 6.4 for *T. phukhaensis* (Pomchote et al., 2020a), 6.75 for *T. verrucosus* (Pomchote et al., 2020b), and 4.7-7.5 for *T. vietnamensis* (Bernardes et al., 2013). For *T. panhai*, Pomchote et al. (2020a) reported a breeding site pH of 6.4. Hernandez et al.

(2019) reported new breeding localities of *T. panhai* at Phu Thap Boek, Lom Kao district, Phetchabun province, north-eastern Thailand with slightly acidic water (pH 6.62). The breeding sites of *T. panhai* at PSDNP were freshwater lentic habitats that ranged from ephemeral puddles to ephemeral small ponds and creeks. The water pH varied within a narrow range of 5 to 6, which is more acidic than that for *T. panhai* at Phu Thap Boek and all the related species except for *T. vietnamensis*. This could be explained by local adaptation of the newts to geographical variations. The geological structure at PSDNP consisted of igneous rock covered with sedimentary rocks (Pongthornpruek et al., 2008). Such aquatic habitats with an igneous bedrock (granite, basalt) typically have a low alkalinity due to the absence of carbonates (Dodd, 2010). In addition, another factor affecting the water pH at breeding sites could be the level of dissolved organic matters, where the clear brown water color with debris from leaves and branches on the waterbed indicate the presence of organic acids (Sherman and Van Munster, 2012), and the decreasing water level would increase their concentration making the water more acidic in the dry season.

Our results on climatic factors are congruent with most other studies, where temperature plays an important role in the adult newts' breeding activities with warmer temperatures increasing the adult newt number in aquatic habitats. Many studies have reported that environmental cues, such as temperature and rainfall, affect the terrestrial life cycle of amphibians (Dervo et al., 2016; Johnson et al., 2017, Todd and Winne, 2006; Vitt and Caldwell, 2013); whereas, there was no relationship between the rainfall level and the number of newts visiting the reproductive sites in this study. Instead, humidity showed a strong relationship with the breeding patterns. Ficetola and Maiorano (2016) reported that temperature appears to have a greater relationship with breeding activities than rainfall across five continents. Moisture is required for amphibians to prevent dehydration when they are on land, such as for reproduction and egg and larval development (Parker, 1960). During the course of this study, we observed female newts attaching their eggs on the base of grasses, mosses growing on rocks, or the soil close to aquatic habitats, which is consistent with the findings of Nishikawa et al. (2013). Thus, a high atmospheric moisture in the wet season may protect the eggs from dehydration during the egg and larval development on land, as well as possibly protecting the eggs from predators in the water (Marangio and Anderson, 1977).

Mark-recapture study of adult *T. panhai*

In this study, we captured more male newts than female newts, resulting in a highly male-biased sex ratio. This could be attributed to the sampling methods. All males were captured using the visual encounter survey method, but four of the 12 females were captured in the three-fence array with pit fall traps. Grayson et al. (2012) indicated a reduced female detection in breeding ponds since they spend more time hidden in the vegetation and substrate to avoid male encounters under a male-biased sex ratio. Thus, the use of the three-fence array with pit-fall traps could increase the efficiency of capturing females. Our study revealed a greater proportion of caught males at the breeding sites during the breeding season, some of which returned to the breeding site in the following year. We detected a lower capture rate of females at breeding sites, which may be due to females skipping one or more years of breeding and remain in terrestrial habitats as females have higher energetic costs for reproduction giving differences in the breeding frequency between sexes (Grayson et al., 2011; Bull and Shine, 1979). In this study, we detected a low estimated population size for *T. panhai* from five sampling occasions. According to the IUCN Red List categorization system for threatened species, the estimated local adult breeding population size of *T. panhai* at PSDNP was more than 50 individuals, which was sufficient for preventing inbreeding depression over five generations in the wild, but with an estimated population size of less than 500 individuals (249 individuals) it still indicated a risk of inbreeding within the population (IUCN, 2001). However, the breeding population size could be underestimated due to the low capture events. Increasing the intensity of the sampling effort and long-term observation would provide a more effective estimated population size and conservation plan for the species at a regional scale.

CONCLUSION

In this study, a local population of *T. panhai* at PSDNP, Thailand was found to be typical seasonal breeders. The cycle of migratory movement in adults at breeding sites was found to be positively correlated with the environmental cues of temperature, humidity, and water pH. Furthermore, water availability and a drought condition influenced the duration that adult newts and larvae stayed in the aquatic habitats. The adult newt population had a small estimated population size with a male-biased sex ratio, though this could reflect a bias in catching efficiencies between the sexes. Females were not only larger overall but also exhibited proportionally larger morphometric traits

than males, indicating sexual size dimorphism in both size and body proportions. No female newts were recaptured across years, in contrast to several recaptured males. This may suggest that females do not breed every year, or that their shorter presence or differing behavior at breeding sites reduces the likelihood of detection and recapture. However, at least some males seem to breed every year. A future framework for conservation actions that benefits the species should include strategies such as protecting and maintaining aquatic breeding habitats, especially during critical breeding seasons; monitoring water availability and quality to mitigate the effects of drought; and implementing long-term population monitoring programs to track sex ratios, breeding frequency, and migratory patterns. Additionally, habitat restoration and the creation of artificial breeding ponds may support population resilience, especially in the face of changing environmental conditions.

LITERATURE CITED

- AmphibiaWeb. 2024. AmphibiaWeb: Information on amphibian biology and conservation. University of California, Berkeley. Available from: <https://amphibiaweb.org>, (September 20, 2025).
- Anderson, J. 1871. Description of a new genus of newts from western Yunan. In Proceedings of the Zoological Society of London, 1871: 423–425.
- Anderson, T.L., Ousterhout, B.H., Peterman, W.E., Drake, D.L. and Semlitsch, R.D. 2015. Life history differences influence the impacts of drought on two pond-breeding salamanders. *Ecological Applications*, 25(7): 1896–1910.
- Bagnouls, F. and Gaussen, H. 1957. Les climats biologiques et leur classification. *Annales de Géographie*, 66: 193–220.
- Bedi, A., Bedi, V., Nag, S. and Suyesh, R. 2021. Wild observations of the reproductive behaviour and first evidence of vocalization in Crocodile newt *Tylototriton himalayanus* (Caudata: Salamandridae) from the Himalayan biodiversity hotspot in Eastern India. *Salamandra*, 57(1): 65–74.
- Bernardes, M., Rödder, D., Nguyen, T.T., Pham, C.T., Nguyen, T.Q. and Ziegler, T. 2013. Habitat characterization and potential distribution of *Tylototriton vietnamensis* in northern Vietnam. *Journal of Natural History*, 47(17–18): 1161–1175.
- Bernardes, M., Rauhaus, A., Michel, C., Nguyen, T.Q., Le, M.D., Pasmans, F. and Ziegler, T. 2017. Larval development and breeding ecology of Ziegler's Crocodile Newt, *Tylototriton ziegleri* Nishikawa, Matsui and Nguyen, 2013 (Caudata: Salamandridae), compared to other *Tylototriton* representatives. *Amphibian and Reptile Conservation*, 11(1): 72–87.
- Böhme, W., Schottler, T., Nguyen, T.Q. and Kohler, J. 2005. A new species of salamander, genus *Tylototriton* (Urodela: Salamandridae), from northern Vietnam. *Salamandra*, 41(4): 215–220.
- Bull, J.J. and Shine, R. 1979. Iteroparous animals that skip opportunities for reproduction. *The American Naturalist*, 114(2): 296–303.
- Dervo, B.K., Baerum, K.M., Skurdal, J. and Museth, J. 2016. Effects of temperature and precipitation on breeding migrations

- of amphibian species in southeastern Norway. *Scientifica*, 2016: 1–8.
- Dodd, C.K. (Ed.). 2010. *Amphibian ecology and conservation: a handbook of techniques*. Oxford University Press Inc., New York, 584 pp.
- Dufresnes, C., and Hernandez, A. 2022. Towards completing the crocodile newts' puzzle with all-inclusive phylogeographic resources. *Zoological Journal of the Linnean Society* 197: 620–640.
- Ficetola, G.F. and Maiorano, L. 2016. Contrasting effects of temperature and precipitation change on amphibian phenology, abundance and performance. *Oecologia*, 181: 683–693.
- Frost, D. R. 2022. *Amphibian Species of the World: an Online Reference*. Version 6.2, American Museum of Natural History, New York, USA. Available from: <https://amphibiansoftheworld.amnh.org/index.php>, (March 1, 2022). doi.org/10.5531/db.vz.0001
- Fu, V.W.K., Karraker, N.E. and Dudgeon, D. 2013. Breeding dynamics, diet, and body condition of the Hong Kong newt (*Paramesotriton hongkongensis*). *Herpetological Monographs*, 27(1): 1–22.
- Geiger, R. 1961. Überarbeitete Neuauflage von Geiger, R.: Köppen-Geiger / Klima der Erde. (Wandkarte 1:16 Mill.), Darmstadt.
- Glime, J.M. and Boelema, W.J. 2017. Amphibians: Anuran adaptations. Chapt. 14-1. In: Glime, J.M. (Ed.). *Bryophyte Ecology. Bryophyte ecology. Volume 2. Bryological Interaction*. Michigan Technological University, Houghton, 1–29.
- Grayson, K.L., Bailey, L.L. and Wilbur, H.M. 2011. Life history benefits of residency in a partially migrating pond-breeding amphibian. *Ecology*, 92(6): 1236–1246.
- Grayson, K.L., De Lisle, S.P., Jackson, J.E., Black, S.J. and Crespi, E.J. 2012. Behavioral and physiological female responses to male sex ratio bias in a pond-breeding amphibian. *Frontiers in Zoology*, 9(1): 1–10.
- Griffiths, R.A. 1986. Feeding niche overlap and food selection in smooth and palmate newts, *Triturus vulgaris* and *T. helveticus*, at a pond in mid-Wales. *The Journal of Animal Ecology*, 55(1): 201–214.
- Halliday, T.R. and Verrell, P.A. 1986. Sexual selection and body size in amphibians. *Herpetological Journal*, 1(3): 86–92.
- Halliday, T. R. and Tejedo, M. 1995. Intrasexual selection and alternative mating behaviour. In: Heatwole, H. and Sullivan, B.K. (Eds). *Amphibian Biology, Social Behaviour*, Surrey Beatty, Chipping Norton, 419–468.
- Hedrick, A.V. and Temeles, E.J. 1989. The evolution of sexual dimorphism in animals: hypotheses and tests. *Trends in Ecology and Evolution*, 4(5): 136–138.
- Hernandez, A. 2016. *Crocodile Newts: The Primitive Salamandridae of Asia: Genera Echinotriton and Tylototriton*. Chimaira Editions, Frankfurt, 415 pp.
- Hernandez, A. 2017. New localities for *Tylototriton panhai* and *Tylototriton uyenoi* Nishikawa, Khonsue, Pomchote, and Matsui 2013 in northern Thailand. *Bulletin de la Société Herpétologique de France*, 162: 110–112.
- Hernandez, A., Escoriza, D. and Hou, M. 2018. Patterns of niche diversification in south-east Asian crocodile newts. *Zoologischer Anzeiger*, 276: 86–93.
- Hernandez, A., Escoriza, D., Pomchote, P. and Hou, M. 2019. New localities for *Tylototriton uyenoi*, *T. panhai* and *T. anguliceps* in Thailand with remarks on the southernmost distribution of the genus. *Herpetological Bulletin*, 147:15–18.
- Hernandez, A., and Pomchote, P. 2020. Habitat, distribution and life history of the polytypic Panha's Crocodile Newt, *Tylototriton panhai* in northeastern Thailand. *Alytes*, 37(3–4): 25–46.
- Hernandez, A., Pomchote, P. and Jamin, A. 2022. First reproduction of Panha's crocodile newt *Tylototriton panhai* in captivity, with a description of the courtship behaviour, eggs and larval development. *Herpetological Bulletin*, 159: 6–11.
- Heyer, W.R., Donnelly, M.A., McDiarmid, R.W., Hayek, L.C. and Foster, M.S. 1994. *Measuring and monitoring biological diversity: standards methods for amphibians*, Smithsonian Institution Press, Washington, 384 pp.
- IUCN. 2001. *IUCN Red List categories and criteria: version 3.1*. Prepared by the IUCN Species Survival Commission. IUCN, Gland, Switzerland and Cambridge, United Kingdom, 30 pp.
- Johnson, E.R., Bowerman, B.L., Thomas, M.A., Thompson, L.M. and Grayson, K.L. 2017. The influence of environmental factors on pond activity of aquatic red-spotted newts *Notophthalmus viridescens*. *Journal of Freshwater Ecology*, 32(1): 711–720.
- Kottek, M., Grieser, J., Beck, C., Rudolf, B. and Rubel, F. 2006. World map of the Köppen-Geiger climate classification updated. *Meteorologische Zeitschrift*, 15(3): 259–263.
- Köppen, W. 1900. Versuch einer Klassifikation der Klimate, vorzugsweise nach ihren Beziehungen zur Pflanzenwelt. *Geographische Zeitschrift*, 6(12): 657–679.
- Kuzmin, S.L., Dasgupta, R. and Smirina, E.M. 1994. Ecology of the Himalayan newt (*Tylototriton verrucosus*) in Darjeeling Himalayas, India. *Russian Journal of Herpetology*, 1(1): 69–76.
- Le, D.T., Nguyen, T.T., Nishikawa, K., Nguyen, S.L.H., Van Pham, A., Matsui, M. and Nguyen, T.Q. 2015. A new species of *Tylototriton* Anderson, 1871 (Amphibia: Salamandridae) from northern Indochina. *Current Herpetology*, 34(1): 38–50.
- Lyu, Z.T., Wang, J., Zeng, Z.C., Zhou, J.J., Qi, S., Wan, H. and Wang, Y.Y. 2021. A new species of the genus *Tylototriton* (Caudata, Salamandridae) from Guangdong, southern China, with discussion on the subgenera and species groups within the genus. *Vertebrate Zoology*, 71: 697–710.
- Maiorana, V.C. 1976. Size and environmental predictability for salamanders. *Evolution*, 30: 599–613.
- Marangio, M.S. and Anderson, J.D. 1977. Soil moisture preference and water relations of the marbled salamander, *Ambystoma opacum* (Amphibia, Urodela, Ambystomatidae). *Journal of Herpetology*, 11: 169–176.
- Nishikawa, K., Khonsue, W., Pomchote, P. and Matsui, M. 2013a. Two new species of *Tylototriton* from Thailand (Amphibia: Urodela: Salamandridae). *Zootaxa*, 3737(3): 261–279.
- Nishikawa, K., Matsui, M. and Nguyen, T.T. 2013b. A new species of *Tylototriton* from northern Vietnam (Amphibia: Urodela: Salamandridae). *Current Herpetology*, 32(1): 34–49.
- Packer, W.C. 1960. Bioclimatic influences on the breeding migration of *Taricha rivularis*. *Ecology*, 41(3): 509–517.
- Phimmachak, S., Aowphol, A. and Stuart, B.L. 2015a. Morphological and molecular variation in *Tylototriton* (Caudata: Salamandridae) in Laos, with description of a new species. *Zootaxa*, 4006(2): 285–310.
- Phimmachak, S., Stuart, B.L., and Aowphol, A. 2015b. Ecology and natural history of the knobby newt *Tylototriton podichthys* (Caudata: Salamandridae) in Laos. *Raffles Bulletin of Zoology*, 63(2015): 389–400.
- Pimentel, R.A. 1960. Inter-and intrahabitat movements of the rough-skinned newt, *Taricha torosa granulosa* (Skilton). *American Midland Naturalist*, 63(2): 470–496.
- Pomchote, P., Khonsue, W., Sapewisut, P., Eto, K. and Nishikawa, K. 2020b. Discovering a population of *Tylototriton verrucosus* (Caudata: Salamandridae) from Thailand: implications for conservation. *Tropical Natural History*, 20(1): 1–15.

- Pomchote, P., Khonsue, W., Thammachoti, P., Hernandez, A., Peerachidacho, P., Suwannapoom, C., Onishi, Y. and Nishikawa, K. 2020a. A new species of *Tylototriton* (Urodela: Salamandridae) from Nan Province, northern Thailand. *Tropical Natural History*, 20(2): 144–161.
- Pomchote, P., Peerachidacho, P., Hernandez, A., Sapewisut, P., Khonsue, W., Thammachoti, P. and Nishikawa, K. 2021. A new species of the genus *Tylototriton* (Urodela, Salamandridae) from western Thailand. *ZooKeys*, 1072: 83–105.
- Pongthornpruek, S., Pampasit, S., Promtep, K. and Nabheerong, P. 2008. The diversity of pteridophytes along a natural trail at Phu Soi Dao National Park, Phitsanulok province, Thailand. *Asia-Pacific Journal of Science and Technology*, 13(8): 895–905.
- Pough, F.H., Andrews, R.M., Cadle, J.E., Crump, M.L., Savitzky, A.H. and Wells, K.D. 1998. *Herpetology* (Vol. 830). Prentice Hall, Upper Saddle River, New Jersey, 577 pp.
- Raffaëlli, J. 2022. *Salamanders & Newts of the World*. Plumelec, France: Penclen Édition.
- R Core Team. 2022. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.
- Schwarz, C.J. and Arnason, A.N. 1996. A general methodology for the analysis of capture-recapture experiments in open populations. *Biometrics*, 52: 860–873.
- Scott, N.J. and Woodward, B.D., 1994. Surveys at breeding sites. Measuring and monitoring biological diversity: standard methods for amphibians. In: Heyer, R., Maureen, A., Donnelly, M., Mercedes, F. and McDiarmid, R. (Eds.). *Measuring and Monitoring Biological Diversity: Standard methods for amphibians*, Smithsonian Institution Press, Washington, 118–125.
- Seglie, D., Roy, D. and Giacoma, C. 2010. Sexual dimorphism and age structure in a population of *Tylototriton verrucosus* (Amphibia: Salamandridae) from the Himalayan Region. *Copeia*, 2010(4): 600–608.
- Semlitsch, R.D. and Wilbur, H.M. 1988. Effects of pond drying time on metamorphosis and survival in the salamander *Ambystoma talpoideum*. *Copeia*, 1988: 978–983.
- Sherman, E. and Van Munster, K. 2012. Pond pH, acid tolerance, and water preference in newts of Vermont. *Northeastern Naturalist*, 19(1): 111–122.
- Shine, R. 1979. Sexual selection and sexual dimorphism in the Amphibia. *Copeia*, 1979: 297–306.
- Smith, D.C. 1983. Factors controlling tadpole populations of the chorus frog (*Pseudacris triseriata*) on Isle Royale, Michigan. *Ecology*, 64(3): 501–510.
- Sparreboom, M. 2014. *Salamanders of the Old World: the salamanders of Europe, Asia and northern Africa*. Royal Dutch Society for Natural History Publishers, Zeist, 431 pp.
- Taylor, E. H. 1962. The amphibian fauna of Thailand. *The University of Kansas Science Bulletin*, 43: 265–599.
- Todd, B.D. and Winne, C.T. 2006. Ontogenetic and interspecific variation in timing of movement and responses to climatic factors during migrations by pond-breeding amphibians. *Canadian Journal of Zoology*, 84(5): 715–722.
- Vitt, L.J. and Caldwell, J.P. 2013. *Herpetology: an introductory biology of amphibians and reptiles*. Academic press, Amsterdam, 720 pp.
- Welsh-Appleby, B. 2015. Analysis of persistent marking techniques using passive integrated transponders and visible im-plant elastomer through metamorphosis in *Ambystoma mavortium*. Environmental Studies Undergraduate Student Thesis, University of Nebraska, Lincoln, 175 pp.
- Whiteman, H.H., Doyle, J.M., Aubee, C., Brown, R., Thomason, S. and Schoborg, T. 2016. A PIT tagging technique for ambystomatid salamanders. *Herpetological Review*, 47: 32–34.
- Wells, K.D. 2010. *The ecology and behavior of amphibians*, University of Chicago press, Chicago (Illinois), 1400 pp.
- Wilcox, J.T., Vang, C.D., Muller, B.R. and Alvarez, J.A. 2017. Drought influences reproductive timing in two newt (*Taricha*) congeners. *Herpetology Notes*, 10: 585–587.