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Ecology and natural history of the knobby newt *Tylototriton podichthys* (Caudata: Salamandridae) in Laos

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Abstract. Almost nothing is known on the ecology and natural history of *Tylototriton* (Knobby Newts) in Laos. Here, a population of the newly described *T. podichthys* was intensively studied in a 55,800 m^2 area in Xiengkhouang Province, Laos, from June 2012–July 2013. Mark-recapture methods estimated 301 individuals of *T. podichthys* at the study site. Newts were abundant during the breeding season (June–July), with a maximum density of 8.75 newts in 100 m^2 of stream. The sex ratio of adult males and females was almost equal and did not differ throughout the year. The population was sexually dimorphic, with females having larger and heavier bodies than males, and different cloacal morphologies during the breeding season. Males, females and immature newts (efts) used a variety of habitat and microhabitat types. Adult newts occupied a stream only during a brief breeding period, but otherwise were primarily terrestrial. The breeding season began in the early rainy season during June–July, and efts emerged from the stream during the dry, cold season beginning in December. Diet was studied by stomach-flushing methods, and consisted primarily of terrestrial invertebrates, especially woodlice (Ligiidae), earthworms (Haplotaxida) and pillbugs (Armadillidae). Unlike in many other newts, conspecific oophagy was not observed. Diet composition, number of prey consumed, and volume of prey consumed did not differ among adult males, adult females or efts.

Key words. amphibian, diet, ecology, life cycle, mark-recapture

INTRODUCTION

The global amphibian decline is a major conservation crisis, and approximately one-third of amphibian species are at risk of extinction (Stuart et al., 2004). Within amphibians, the Order Caudata (salamanders) has the highest proportion of threatened species (Hilton-Taylor et al., 2009). The primary cause of the global amphibian decline is habitat loss, and in Southeast Asia, the most needed conservation actions for amphibians are identification and strict protection of habitat (Rowley et al., 2010). Habitat use, life history, and ecology of a species must be known to effectively conserve it (Taylor et al., 2006). Asian members of the caudate family Salamandridae ("newts") are additionally threatened by overexploitation for traditional medicine and the international pet trade (Rowley et al., 2010; Phimmachak et al., 2012; Sparreboom, 2014; Stuart et al., 2014; Nishikawa et al., 2014).

The genus *Tylototriton* ("Knobby Newts") has a wide geographic distribution, ranging from the eastern Himalayas through central China, and southward into northern parts of Myanmar, Thailand, Laos and Vietnam. Approximately 20 species of *Tylototriton* are currently recognised (Shen et al., 2012; Hou et al., 2012; Nishikawa et al., 2013a, b, 2014; Yang et al., 2014; Frost, 2015; Phimmachak et al., 2015). *Tylototriton* taxonomy is currently in flux, as many new species have recently been described, and species boundaries are uncertain (Stuart et al., 2010; Yuan et al., 2011; Zhang et al., 2013; Nishikawa et al., 2014).

Most information on the ecology and natural history of *Tylototriton* is based on *T. verrucosus* in South Asia (India, Nepal, and Bhutan), including its general ecology (Kuzmin et al., 1994), sexual dimorphism and population structure (Seglie et al., 2010), feeding and reproduction (Dasgupta, 1996, 1997), and courtship behaviour (Roy & Mushahidunnabi, 2001).

Additional information on the natural history and ecology of other *Tylototriton* species includes general ecology of Chinese taxa (Yu & Zhao, 2005), habitat use of *T. asperrimus* (Sun et al., 2011) and *T. vietnamensis* (Bernardes et al., 2013), age assessment of *T. panhai* (Khonsue et al., 2010), and reproduction in wild and captive *T. kweichowensis* (Tian et al., 1998), *T. wenxianensis* (Gong & Mu, 2008), and *T. shanjing* (Ziegler et al., 2008; Jun et al., 2012).

The amphibian fauna of Laos is relatively poorly known. The genus *Tylototriton* was first reported from Laos with the description of *T. notialis* from Khammouan Province (Stuart et al., 2010). A second species, *T. podichthys* Phimmachak, Aowphol & Stuart, 2015, was recently described from Luang Phabang, Xiengkhouang and Huaphanh Provinces (reported as *T. shanjing* in Matsui, 2013; Nishikawa et al., 2013b), and is currently considered to be endemic to northern Laos

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(Phimmachak et al., 2015). *Tylototriton* are harvested in Laos for the international pet trade and traditional medicine (S. Phimmachak, pers. obs.). *Laotriton laoensis*, another newt species that has a very similar geographic range to *T. podichthys*, is listed as Endangered in the IUCN Red List of Threatened Species, primarily owing to overharvesting (Stuart et al., 2006; Phimmachak et al., 2012; IUCN, 2014). It is therefore very possible that *Tylototriton* is also threatened in Laos. The *T. verrucosus* species complex in adjacent Vietnam has recently disappeared from parts of its range (Nguyen et al., 2009; van Dijk et al., 2009; Bernardes et al., 2013). The loss of these newts in Vietnam only strengthens the need to conserve wild *Tylototriton* in Laos while the opportunity exists. Information is urgently needed on the ecology and natural history of *T. podichthys* in Laos, and that is the basis of this study.

MATERIAL AND METHODS

Focal study site. The ecology and natural history of *T. podichthys* were intensively studied within an approximately 55,800 m^2 area (Fig. 1) surrounding Houay Mor Stream at Yord Lieng Village, Kham District, Xiengkhouang Province, Laos (19°50’23.9” N 103°42’11.5” E, ca. 1,503 m elevation) from June 2012 to July 2013. The site had been used for agricultural purposes, including rice and livestock production, for the previous 25 years. The site contained three distinct habitat types, classified as forest (trees >5 m in height; 29,800 m^2), shrub forest (trees ≤ 5 m in height; 26,000 m^2) (Lenard, 2008; FAO, 2010), and a temporary stream (400 m^2) that was 0.5–3 m wide with some pools, 200 m in length, and flowed through shrub forest and forest.

Sampling and data collection. Daytime visual searching was conducted during four periods, totaling 29 days between June 2012 and July 2013 (28 June–6 July 2012; 21–25 August 2012; 10–17 December 2012; 1–7 July 2013). These sampling periods represented both wet and dry seasons at the study site. Newts were captured by hand and dipnet. Five pitfall traps, each consisting of 10 m of plastic fencing with two buckets recessed to ground level, were positioned on land 5–20 m from the stream bank to capture newts and terrestrial insects during the sampling periods. Data were recorded for each captured newt at the site of capture, including habitat, substrate type, air temperature, stream water temperature, stream water pH, and air relative humidity using pH paper and a Kestrel 3500 Pocket Weather Meter. Meteorological data for each sampling period is shown in Table 1. Temperature and rainfall data were taken from the Kham Meteorology Station (Lao Department of Meteorology) during June 2012–July 2013 (Fig. 4). All captured newts were temporarily housed in captivity (≤24 h) for flushing stomach contents, taking morphological measurements, and marking individuals (below).

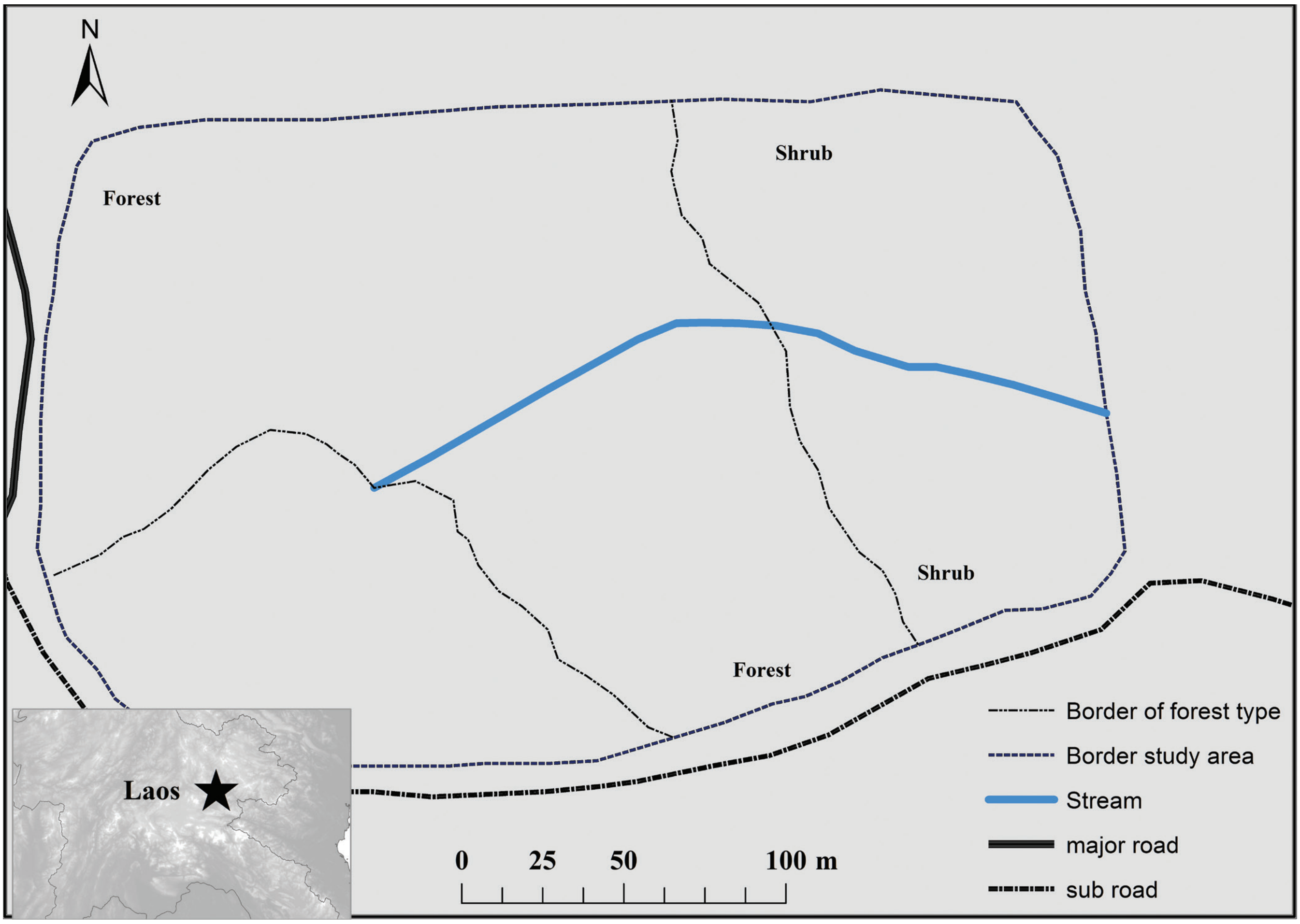

Fig. 1. Study site of *Tylototriton podichthys* at Houay Mor Stream, Yord Lieng Village, Kham District, Xiengkhouang Province, Laos.

Table 1. The mean ± standard deviation (SD) of air temperature (AT) and water temperature (WT), pH and relative humidity (H) during each sampling period.

	AT(°C)	WT(°C)	pH	H (%)
June 2012	23.0 ± 2.0	20.0 ± 0.3	6.0	96.0 ± 4.1
August 2012	22.7 ± 0.8	19.7 ±1.6	6.0	96.7± 2.2
December 2012	18.8 ± 2.7	14.8 ±1.9	5.5	93.0 ± 5.0
July 2013	23.6 ± 2.0	19.0± 1.1	6.0	93.9 ± 4.3

Population structure. Newts were sexed using external cloacal characters (Shen et al., 2012; Fig. 2). Accuracy was verified by dissecting the reproductive tracts of two males and two females from the study site during June 2012. Newts lacking sexual cloacal characters were classified as immature, and sub-classified as either subadult (immature aquatic stage) or eft (immature terrestrial stage). The numbers of individuals of males, females, and immature newts were recorded during each sampling period. Sex ratios of newts during each sampling period and throughout the year (June 2012–July 2013) were compared using contingency table analysis and the Chi-square Goodness of Fit test.

Adult population size and density. Adult population size at the study site was estimated from data obtained during two sampling periods (June–July 2012 and August 2012) using the Lincoln-Peterson (Chapman) formula (Chapman, 1951). Immigration, emigration and death of newts were not found in the study site during the two sampling periods (June–August 2012). Newts were immersed in a solution of the anesthetic tricaine methane sulfonate (MS-222; 1 g/l) for less than ten minutes (Leclerc & Courtois, 1993). All adult and large immature (SVL > 45 mm) newts were permanently and uniquely marked using 8.0 mm PIT tags (Biomark, Inc.) into the body cavity. Smaller immature newts were marked by toe clipping following Donnelly et al. (1994). Toe clipping only aided identifying individuals during the sampling period, but not between sampling periods, as the newts re-grew clipped toes in approximately two weeks. Anesthetised newts were rinsed in fresh water to regain consciousness, maintained in captivity for 24 hours to allow recovery, and released at the point of capture.

Fig. 2. Sexual dimorphism of *Tylototriton podichthys* at the study site. A, Dorsal view of adult female; B, dorsal view of adult male; C, ventral view of female cloaca; D, ventral view of male cloaca.

Abundance (Krebs, 1999) was calculated as absolute density (newts/100 m^2) of newts including all newt groups (females, males, immature newts) in three habitat types (forest, shrub and stream). The absolute density was calculated from the actual number of newts encountered during each sampling period divided by the area of each habitat type.

Sexual dimorphism. The following measurements were taken from adult newts to the nearest 0.1 mm with dial calipers: SVLA = snout–vent length from tip of snout to anterior edge of vent; SVLP = snout–vent length from tip of snout to posterior edge of vent, TTL = total length; and CL = the length from anterior cloacae to posterior cloacae (CL= SVLA-SVLP). Newts were weighed with an electronic balance. Morphometric data were tested for normality using Shapiro-Wilk's test and morphological differences between sexes were compared with a t-test using XL-Stat 2014.3 (Addinsoft).

Habitat use. Air temperature, water temperature and relative humidity were recorded for each observed individual. Newts were searched in all shelters in the study site. Habitat classifications (forest, shrub forest and stream) and microhabitat descriptions were also recorded for each individual. Those microhabitats were categorised into six substrate types on land and in the dry stream: (1) under grasses on land; (2) under logs on land; (3) under leaf litter on land; (4) under grasses in the stream; (5) under logs in the stream, and (6) on the surface of the ground. Chi-square tests of independence were performed to determine percentages of frequency and association between sex (female, male, immature), observation period, and habitat used. The distance from water (m) for each group (adult male, adult female, immature newt) in the four observation periods was compared using the non-parametric Kruskal-Wallis test on XL-Stat 2014.3 (Addinsoft).

Life cycle. Oviposited eggs and larvae were actively searched for in the stream during June 2012–July 2013. Efts were actively searched for on the ground adjacent to the stream and in the forest at the study site by raking leaf litter and lifting logs and other ground cover. A total of 62 eggs were obtained from seven females found near the stream that oviposited while being housed overnight in captivity. One marked female was kept in a plastic aquarium for eight days before release at the site of capture to verify that the PIT tag was retained and did not harm newt health. Reproductive tracts were dissected from two adult males and two adult females at the study site in early July 2012. Voucher specimens were fixed in 10% formalin, subsequently preserved in 70% ethyl alcohol and deposited in the herpetological collection, Department of Biology, National University of Laos, Vientiane, Laos (NUoL 00435–00436, NUoL 00444–00445).

Growth rate. Growth rates of newts were estimated using SVLA only, as body mass was affected by stomach contents at time of capture. Body length (SVLA) of each individual was measured at the initial capture and each time of recapture. Monthly growth rates were estimated from capture and recapture samples during the four study periods using linear regression analysis in XL-Stat 2014.3 (Addinsoft).

Diet. Stomach contents of the newts were sampled by flushing the stomach, following Denoel et al. (2007). Stomach contents were fixed in 10% buffered formalin and later transferred to 70% ethanol before being examined with a stereomicroscope.

Prey availability on land at the study site was determined by collecting invertebrates at 28 randomly placed quadrats of 25 cm × 25 cm on 28 August 2012 and 11 December 2012, and from five pitfall traps set for 8 days in June 2012 and 8 days in December 2012. Sampled invertebrates were preserved in 70% alcohol, and species diversity and abundance were determined. Stomach contents and sampled invertebrates were identified to at least family using Chu & Cutkump (1992), Malaipan (1999) and Triplehorn & Johnson (2005).

Number and volume (mm^3) of prey consumed in stomachs were calculated following the formula from Griffiths (1986). Index of Relative Importance (IRI) of each prey type was calculated (Pinkas et al., 1971) using the formula IRI = %Fi (%Ni +%Vi), where %Ni is the percentage number of prey type i (relative to total number of prey), and %Vi and %Fi are the percentage volume and the percentage frequency of occurrence of prey type, respectively. The Shannon-Wiener Index (H′) was used to calculate prey diversity in stomach contents. Niche breadth (Krebs, 1999) was calculated for each newt group in each sampling period using Levins's niche breadth index $B = 1/\sum (p_j)^2$ in its standardised B′ = (B-1)/(n-1), where p_j is the proportion of prey of type j found in a particular newt group's diet during each sampling period, and n is the number of all prey types. Correlation between volume of prey consumed and body size (SLVA) was tested using Spearman's rank correlation. The non-parametric Kruskal-Wallis test was used to compare number and abundance of species in the stomach contents of newts in each group.

RESULTS

Population structure. A total of 380 individuals (107 males, 122 females, 151 immature newts) were sampled in the 55,800 m^2 study site at Houay Mor during the four sampling periods. A total of 17 individuals were recaptured. The population structure during June 2012–July 2013 is shown in Fig. 3. Newts were most often encountered during the rainy season. During the first and fourth sampling periods (beginning of wet season), 101 and 196 individuals were found, respectively. During the second sampling period (middle of wet season), 68 individuals were found and during the third sampling period (dry season), only 25 individuals were found (Fig. 4). The sex ratio (male: female) did not significantly different among the four sampling periods (1.1:1; $x^2 = 0.325$; $P > 0.05$) but during the third sampling period (December 2012), a higher ratio of males than females (1.8:1; $x^2 = 1.47$; $P > 0.05$) was found.

Population size and density. A total of 62 individuals (30 males, 32 females) were marked during the first sampling

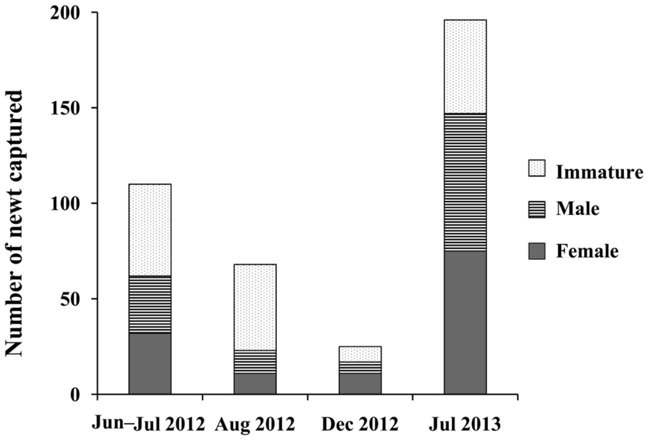

Fig. 3. Seasonal population structure of *Tylototriton podichthys* at the study site. Newts were captured by visual searching and pitfall traps during four sampling periods from June 2012 to July 2013.

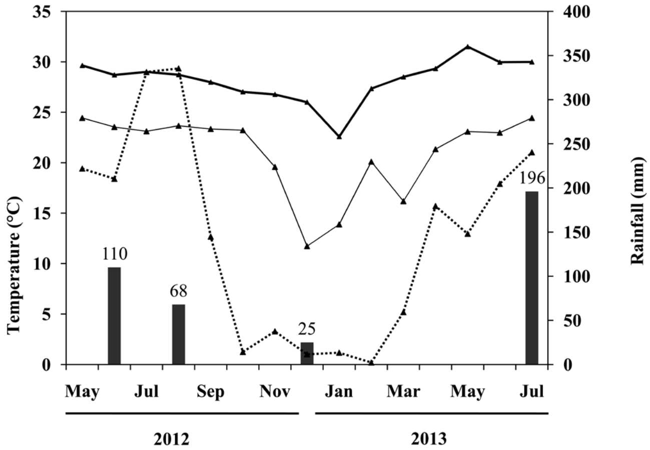

Fig. 4. Temperature, rainfall, and number of captured newt during each sampling period from June 2012 to July 2013. The black bars represent the number of newts, the full bold line represents mean maximum temperature, the full line represents mean minimum temperature, and the dashed line represents rainfall.

period in June–July 2012. A total of 23 individuals (12 males and 11 females) were marked during the second sampling period in August 2012, and four of these (two males, two females) were recaptures from the first sampling period. The estimated population size (N) at the 55, 800m^2 study site of all newts was 301.4 ± 105.4 (Mean ± S.E.; 95% confidential interval [CI] = 281.2–321.6), of males was 133.3 ± 56.0 (95% CI = 118.6–148.0), and of females was 131.0 ± 54.5 (95% CI = 281.2–321.6).

The newts were most abundant (= most easily detected) during the breeding season (June–July), with a maximum density of 8.75 newts in 100 m^2 of stream (Table 2). Newt density decreased outside of the breeding season in all habitat types (Table 2). The lowest density was found in December (dry season), which was 0.06 newts/100 m^2 in the forest and 0.03 newts/100 m^2 in shrub forest, and none in the stream.

Sexual dimorphism. Adult males and females significantly differed in all five morphological characters ($P < 0.01$). Adult females had larger SVLA, SVLP, TTL and body weight than did males (Table 3). Females were more robust and had noticeably larger abdomens during the breeding season (June–July). Cloacal differences between the sexes were more conspicuous during the breeding season and male cloacae is larger than female (Fig. 2).

Habitat use. Based on 380 individuals captured and 17 individuals recaptured during the four sampling periods, it was estimated that 32.3% of females, 29.6% of males, and 38.1% of the immature newts at the study site were sampled. All groups were found in all three habitat types, with 40.9%, 42.9%, and 16.3% in forest, shrub forest, and

Table 2. Estimated densities (newts/100 m^2) of populations of *Tylototriton podichthys* during four sampling periods in three habitat types.

Habitat type	June–July 2012	August 2012	December 2012	July 2013
Forest	0.11	0.12	0.06	0.25
Shrub forest	0.18	0.12	0.03	0.33
Stream	7.50	0.00	0.00	8.75

Table 3. Mean and standard deviation of morphological measurements of adult male and female *Tylototriton podichthys* at the study site. *P*-values are from *t*-tests (**indicates a significant difference of less than $P < 0.001$). Abbreviations are defined in the text.

Measurement	Males (n = 107)		Females (n = 122)		*P*-value
	Mean	SD	Mean	SD	
Weight (g)	7.9	1.2	14.9	2.8	< 0.001**
SVLA (mm)	60.7	3.5	74.7	4.2	< 0.001**
SVLP (mm)	66.3	3.9	78.4	4.4	< 0.001**
Cloacae (mm)	5.6	1.2	3.8	1.3	< 0.001**
Cloacae/TTL	0.026	0.009	0.045	0.009	<0.001**
TLL (mm)	125.3	9.6	147.2	9.7	< 0.001**

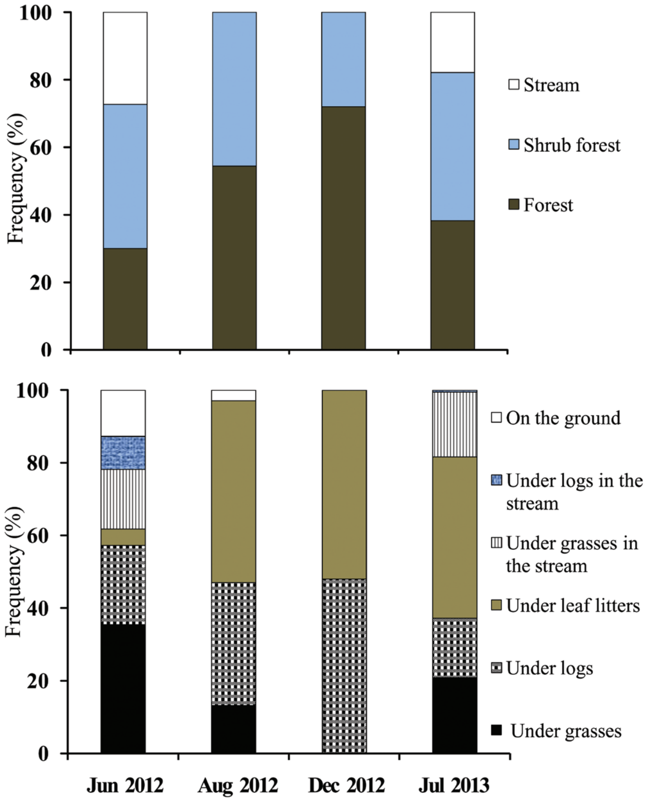

Fig. 5. A, Percentages of habitat types; and B, microhabitat types of *Tylototriton podichthys* at the study site during four sampling periods.

stream, respectively. Occurrences in each habitat type are shown in Fig. 5A. Only larvae (and not immature and adult newts) were found in the stream between August and December 2012. Newts utilised multiple microhabitats. Percentages of newts found on substrate types throughout the year were 34.8% under leaf litter on land, 22.8% under logs on land, 22.3% under grasses on land, 13.3% under grasses in stream, 2.8% under the logs in stream, and 4% on ground surface. Most found on the ground surface were actively moving during the wet season. The first and fourth periods were the breeding season of this species. Significant relationships were found during the first and fourth sampling periods between newt group and habitat type (first period: $x^2 = 20.5$; df = 4; $P = 0.0004$, fourth period: $x^2 = 10.3$; df = 4; $P = 0.036$) and between newt group and microhabitat type (first period: $x^2 = 28.3$, df= 10, $P = 0.001$; fourth period: $x^2 = 19.1.3$, df = 10, $P = 0.001$). No significant relationships were found during the second and third sampling periods between newt group and habitat type (second period: $x^2 =5.7$, df = 2, $P = 0.58$; third period: $x^2 =5.7$; df = 2; $P = 0.58$) and newt group and microhabitat type (second period: $x^2 = 9.2$, df = 6, $P = 0.15$; third period: $x^2 = 2.43$; df = 2; $P = 0.4$).

All groups of newts were found far from the stream outside of the breeding season. During the breeding season, males and females moved closer to the stream, as shown by significant differences between distance to water and newt groups during the first and fourth sampling periods. Mean distance to water of females, males and immature newts were 7.6 ± 14.0, 19.2 ± 24.0 and 13.5 ± 19.1m (mean ± SD), respectively, ($x^2 = 6.88$; $P = 0.03$) during the first sampling period, and 29.1 ± 32.7, 44.0 ± 35.3 and 45.9 ± 35.9m (mean ± SD), respectively, ($x^2 = 8.32$; $P = 0.02$), during the fourth sampling period. No significant differences between distance from water and of all newt groups were found during the second and third sampling periods. Mean distance to water of females, males and immature newts were 59 ± 18.6, 42.0 ± 21.8 and 57.8 ± 16.3m (mean ± SD), respectively ($x^2 =5.36$; $P =0.07$), during the second sampling period, and 61.5 ± 22.7, 53.3 ± 16.3 and 64.2 ± 25.9m (mean ± SD), respectively ($x^2 = 1.44$; $P = 0.49$), during the third sampling period.

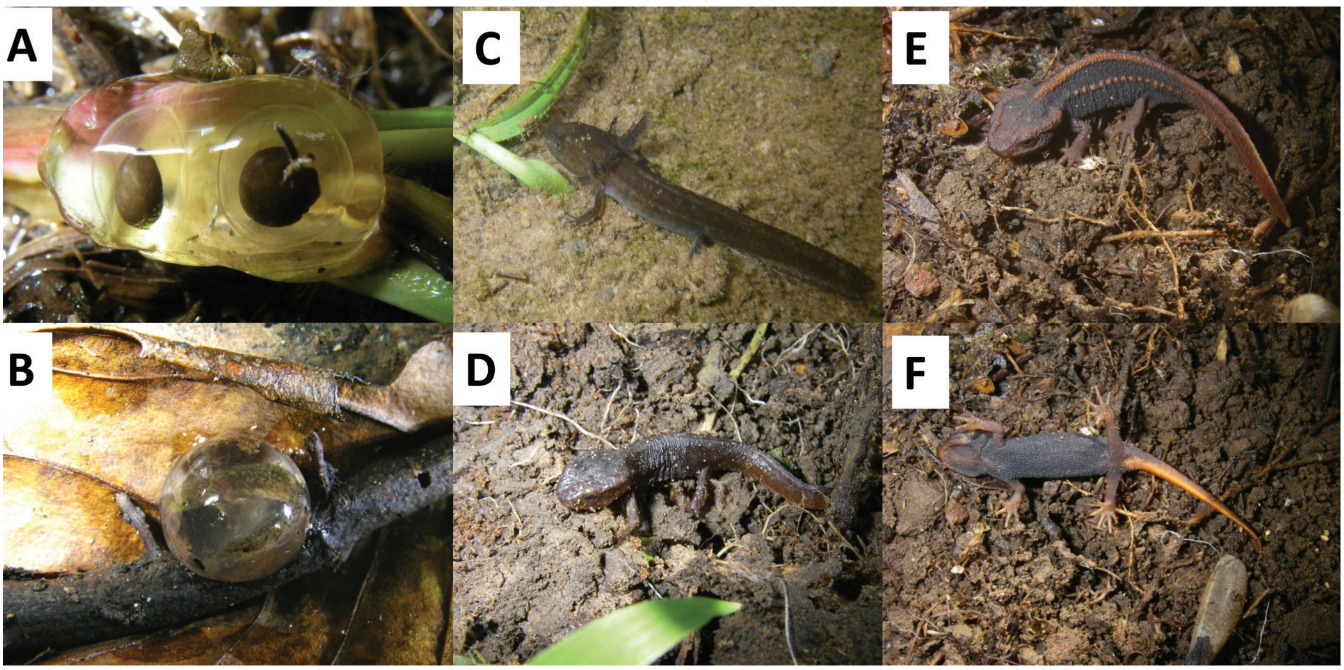

Fig. 6. Life stages of *Tylototriton podichthys* at the study site. A, Eggs under grass in June; B, eggs on leaf litter in July, C, larvae at bottom of stream pool in October, D, eft emerging onto land, found under a log 30 cm from the stream water on 10 December 2012, E, dorsal view; and F, ventral view of an eft that has begun to resemble an adult newt.

Table 4. Mean and standard deviation (SD) of morphological measurements and weights of efts of *Tylototriton podichthys* at the study site. Abbreviations are defined in the text.

Measurement	Emergent Efts in December (n=8)		All Other Efts (n=143)	
	Mean	SD	Mean	SD
Weight (g)	0.7	0.1	2.9	1.9
SVLA (mm)	27.2	2.6	42.6	7.0
SVLP (mm)	29.6	3.1	45.8	8.0
TLL (mm)	55.4	8.5	82.3	17.3

Life cycle. The breeding season of newts at the study site began in the early rainy season (June–July). However, courtship and mating behaviour was not observed during the study. In June–July 2012, male and female newts moved to the dry stream during rainy days and nights. Females deposited their eggs under thick grasses. Oviposited eggs were adhered to the grasses (leaves, trunk, root), leaf litter, and dead branches in single to three eggs (Fig. 6). Developing eggs were found in the dry grassy stream (no water). Early stage eggs were sticky and round in shape. Eggs were dark cream with a dark brown pole and enclosed in a clear jelly capsule. Some eggs had completed development (but not yet hatched) before the stream filled with rainwater and began flowing.

Females maintained temporarily in captivity released four to 12 eggs per night. Newly oviposited eggs were 2.9 ± 0.2 mm (mean ± SD), and eggs with the jelly capsule were 5.0 ± 0.3 mm. Two females that were captured on 6 July 2012 on land 60–70 meters from the stream, had eggs in their ovaries but none in the oviducts.

By early July, the stream had filled with water, and a few larvae were found in the bottom of grassy stream pools for a few days. Larvae without legs had three pairs of gills, yellow-brown body colouration, and dark brown spots on the head, dorsal surface of gills, and dorsal and ventral tail fins. These larvae had total lengths of 12.7 ± 0.8 mm (mean ± SD; n = 8). In August, no eggs but many larvae were found in stream pools. During this sampling period, larvae had developed a longer body, four legs, and three pairs of gills. These larvae had total lengths of 24.3 ± 7.6 mm (mean ± SD; n = 14). Larvae were last observed during the December sampling period. These larvae had total lengths of 35.9 ± 5.1 mm (n = 10, mean ± SD; Fig. 7).

Efts were found during all four sampling periods. The smallest efts (apparently recently emerged) were found during the December sampling period (Fig. 6 D). These efts had total lengths of 55.4 ± 8.5 mm (mean ± SD; n = 8) with body weights of 0.7 ± 0.1 g (mean ± SD; n = 8). Body sizes of all efts found are shown in Table 4. One eft (PIT tag number 96951208) was first sampled in August 2012, and based on its body size, was born during the previous breeding season. This individual was recaptured in June 2013, and did not yet exhibit mature characteristics at presumably two years of age.

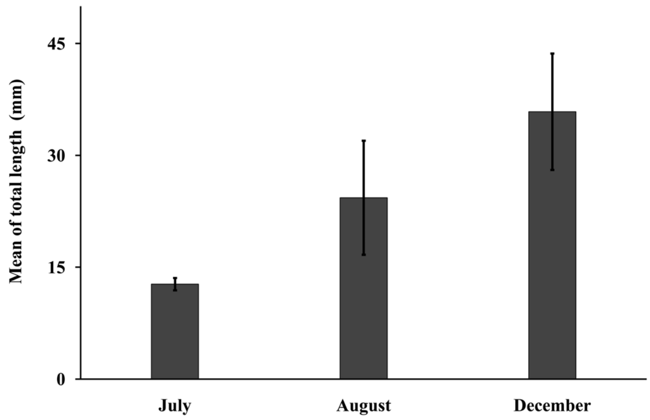

Fig. 7. Means and standard deviations of total lengths of wild larvae of *Tylototriton podichthys* at the study site during July to December 2012.

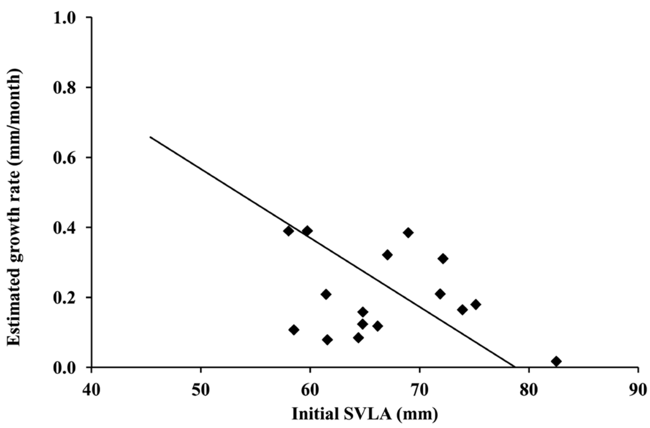

Fig. 8. A comparison of initial body size (SVLA) with monthly growth rates of *Tylototriton podichthys* at the study site, based on 17 recaptured individuals during the study. Growth rate and initial SVLA had a significant negative correlation (r^2 =0.42; P<0.01).

Estimated growth rate. A total of 17 marked individuals were recaptured during the last three periods (the second, third and fourth sampling periods). Estimated monthly growth rates (SVLA) of adult females were 0.20±0.13 mm (mean ± SD; n = 8), of adult males were 0.21±0.12 mm (mean ± SD; n = 8) and of the single recaptured eft was 1.14 mm. A comparison of initial body length (SVLA) with estimated monthly growth rate is shown in Fig. 8. The linear regression calculated for newt growth rate was $y = 1.551 - 0.019x$, (n = 17), $r^2 = 0.42$. The initial SVLA and estimated monthly growth rate had a negative significant correlation (P = 0.005). Smaller individuals grew at a faster rate than

Table 5. Diet composition of *Tylototriton podichthys* at the study site during four sampling periods from June 2012–July 2013. %N = the percentage number of prey type (relative to total number of prey), %F = the percentage frequency of occurrence of prey type, %V= the percentage volume of prey type, and %IRI = the percentage of Index of Relative Importance.

Order	Prey types	% N	% F	% V	% IRI
Archaeognatha	Meinertellidae	0.5	0.3	0.2	0.0
	Microcoryphia	0.2	0.3	0.1	0.0
Araneae	Lycosidae	0.5	0.6	0.2	0.0
Blattodea	Blaberidae	0.7	0.8	2.0	0.1
Coleoptera	Carabidae	0.7	1.1	0.2	0.0
	Curculionidae	0.2	0.3	0.2	0.0
	Staphylinidae	1.6	1.9	1.4	0.3
	Scarabaeidae	0.9	1.1	0.7	0.1
	Tenebrionidae	0.2	0.3	0.2	0.0
Dermaptera	Anisolabididae	3.2	2.8	1.7	0.7
	Forficulidae	0.5	0.3	0.2	0.0
	Pygidicranidae	0.9	0.8	0.6	0.1
Diptera	Tipulidae	0.2	0.3	0.1	0.0
Hemiptera	Lygaeidae	0.5	0.6	0.7	0.0
	Reduviidae	0.2	0.3	0.4	0.0
	Scutelleridae	0.2	0.3	0.2	0.0
Hymenoptera	Formicidae	1.1	1.4	0.2	0.1
Isopoda	Armadillidae	8.7	8.0	7.5	6.6
	Ligiidae	30.3	27.0	11.9	58.3
Julida	Julidae	2.3	2.2	0.9	0.4
	Parajulidae	4.1	4.7	2.9	1.7
Lepidoptera	Not identified (worm)	4.1	4.7	3.5	1.8
Lithobiomorpha	Lithobiidae	6.2	6.9	4.4	3.7
Architaenioglossa	Cyclophoridae	5.1	3.0	4.1	1.4
Haplotaxida	Not identified (earthworm)	6.9	8.0	34.1	16.8
Opiliones	Phalangiidae	0.5	0.6	0.7	0.0
Orthoptera	Gryllidae	0.7	0.8	0.7	0.1
Polydesmida	Paradoxosomatidae	1.6	1.9	2.1	0.4
	Polydesmidae	1.6	1.9	2.7	0.4
	Pyrgodesmidae	0.9	0.8	0.5	0.1
Scolopendromorpha	Scolopendridae	0.9	1.1	0.5	0.1
Scutigeromorpha	Scutigeridae	0.5	0.3	0.5	0.0
Sphaerotheriida	Sphaerotheriidae	0.9	1.1	1.2	0.1
Stylommatophora	Helicarionidae	0.7	0.8	1.6	0.1
	Hygromiidae	2.1	2.5	1.4	0.4
	Subulinidae	1.6	1.9	1.3	0.3
	Zonitidae	7.1	7.7	7.2	5.7

Table 6. Mean ± standard deviation (SD) of consumed prey, niche breadth, prey volume, number of each prey type, and effective number of species calculated using the Shannon-Wiener Index [Exp(H')] in stomach contents of *Tylototriton podichthys* at the study site during four sampling periods.

Period	Group	n	Number of Prey Items	Niche Breadth	Prey Volume	Species Richness	Exp(H′)
Jun 2012	Female	19	1.6±0.7	7.54	94.7±82.5	18	12.5
	Male	25	1.8±1.7	8.33	279.4±565.5	16	11.4
	Immature	42	1.7±0.9	5.112	170.8±370.9	18	9.3
Aug 2012	Female	10	1.5±0.9	8.33	124.9±101.7	10	9.1
	Male	7	1.8±1.0	5.14	264.0±161.1	7	6.0
	Immature	37	1.9±0.9	4.95	125.9±111.7	18	9.1
Dec 2012	Female	7	1.4±0.8	6.25	72.3.9±47.0	7	6.6
	Male	3	1.3±0.6	4.00	104.6±23.4	4	4.0
	Immature	4	1.8±1.0	1.82	142.7±109.2.8	3	2.2
Jul 2013	Female	35	1.7±1.0	9.62	145.6±141.5	19	13.0
	Male	30	2.0±2.4	10.01	152.8±237.5	22	14.5
	Immature	37	1.6±0.8	5.33	96.9±90.0	14	7.9

did larger individuals. The fastest monthly growth rate was that of the single eft that grew 1.14 mm, or 2.5% of initial SVLA, and the slowest growth rate was that of the largest female that grew only 0.02 mm, or 0.02% initial SVLA.

Diet. Stomachs were flushed from 380 captured newts and 17 recaptured newts. Of these, 254 (63.7%) contained at least one prey item. The 254 individuals with stomach contents included 71 (17.8%) females, 65 (16.3%) males and 118 (29.6%) immature newts. A total of 435 prey items were identified at least to family and were grouped into 38 categories (Table 5).The diet composition of the newt populations consisted of insects, earthworms and other arthropods such as woodlice, centipedes, and millipedes. Plant fragments i.e., leaf litter and small pieces of logs were found in 37 newts, but these were assumed to be accidentally ingested during prey capture and were not included in the analyses. The most frequent diet categories (Table 5) were woodlice in the Family Ligiidae with Index of Relative Importance (IRI) = 1139.9 (58.3%), earthworms in Order Haplotaxida with IRI = 327.6 (16.8%), and pill bugs in the Family Armadillidae with IRI = 129.9 (6.6%). Ligiidae also represented the highest prey availability at the study site (Table 7). No significant correlation was found between SVLA and volume of consumed prey ($r = -0.014$; $P = 0.82$; n = 254) among all newt groups. No significant differences were found between newt groups in the number of prey items ($x^2 = 0.86$; $P = 0.65$), prey abundance ($x^2 = 0.28$; $P = 0.96$), or prey volume ($x^2 = 3.7$; $P = 0.16$). The most important food for immature newts was woodlice in family Ligiidae. Niche breadth standardised of newt groups ranged from 0.23 to 1.0 from all four sampling periods, with the widest niche breadth in males (Table 6).

DISCUSSION

Tylototriton podichthys was found to be abundant during the rainy season in June–July at the study site in Kham District, Xiengkhouang Province, Laos. The early rainy season is the most active period of the year for breeding and feeding at this locality. Newts were infrequently encountered during the dry, cold season in December–January, suggesting that they aestivate during this part of the year. This pattern is similar to that observed in the closely-related *T. verrucosus* in the Indian subcontinent, which emerges from hibernation at the beginning of the monsoon season, and is active only during the rainy season in May–October (Seglie et al., 2003; Wangyal & Gurung, 2012).

Sex ratio of *T. podichthys* at the study site did not differ throughout the year, but at Ailao Mountain, Yunnan Province, China, more males of *T. shanjing* were encountered in the early and late parts of the breeding season, with more females encountered during the middle of the breeding season (Jun et al., 2012). Three populations of *T. verrucosus* in Bhutan were observed in July and revealed a higher tendency of males at two localities (Toebisa & Kazhi), but equal sex ratio was found at one locality in Kabjisa (Wangyal & Gurung, 2012).

Adults of *T. podichthys* are consistent with 61% of other caudates by exhibiting sexual size dimorphism (SSD) (Shine, 1979; Labus et al., 2013). Females are larger and heavier than males. Males have a longer cloacal ditch than females, as also seen in the salamandrids *T. verrucosus*, *Triturus cristatus* and *Lissotriton vulgaris* (Malmgren & Thollesson, 1999; Seglie et al., 2010).

Tylototriton podichthys at the study site altered habitat use throughout the year. During the dry season, the newts sheltered under logs and leaf litter in the forest, probably owing to the humidity offered by the closed canopy. This was also observed in a population of *T. shanjing* in Yunnan Province, China that inhabited a moist, shady forest (Grosse, 2007). Other than a brief courtship and spawning period at the beginning of the rainy season, *T. podichthys* were much more terrestrial than aquatic. During the cold,

Table 7. Percentage (%) of sampled invertebrates (potentially available prey for *Tylototriton podicthys*) at the study site.

Order	Family	%	Order	Family	%
Araneae	Agelenidae	6.7	Hemiptera	Pentatomidae	1.1
	Clubionidae	0.9		Reduviidae	0.1
	Dysderidae	0.1	Hymenoptera	Formicidae	8.1
	Lycosidae	1.4	Isopoda	Armadillidae	3.0
	Segestriidae	2.0		Ligiidae	17.9
Archaeognatha	Meinertellidae	1.4		Terimitidae	0.1
Architaenioglossa	Cyclophoridae	0.6	Julida	Julidae	1.1
Arthopoda	Gryllidae	1.9		Parajulidae	1.0
Blattodea	Blattidae	0.1	Lithobiomorpha	Lithobiidae	2.3
	Blaberidae	1.6	Mantodea	Not identified	0.2
Callipodida	Abacionidae	0.5	Mesostigmata	Mantidae	0.1
Coleoptera	Carabidae	9.1	Opiliones	Phalangiidae	4.6
	Cerambycidae	0.1		Phalangiidae	0.1
	Cryptophagidae	0.3	Orthoptera	Gryllidae	3.8
	Curculionidae	0.9		Gryllacridedae	0.2
	Dytiscidae	0.2		Rhaphidoridae	1.5
	Larvae of Coleoptera	1.5		Tetrigidae	0.3
	Lathridiidae	0.2	Phasmida	Scolopendridae	1.1
	Scarabaeidae	2.5	Poduromorpha	Poduridae	0.9
	Staphylinidae	6.0	Polydesmida	Polydesmidae	0.4
Dermaptera	Anisolibididae	1.5		Paradoxosomatidae	0.5
	Forficulidae	0.7	Scolopendromorpha	Scutigeridae	1.2
	Labiidae	0.3	Scutigeromoroha	Sphaerotheriidae	1.3
	Pygidicranidae	0.1	Stylommatophora	Camaenidae	0.1
Diptera	Drosophilidae	0.3		Helicarionidae	0.1
	Tipulidae	0.3		Hygromiidae	0.8
Entomobryomorpha	Entomobryidae	0.3		Not identified	0.1
Hemiptera	Hebirdae	2.3		Subulinidae	0.6
	Lygaeidae	0.4		Zonitidae	2.7
	Nabidae	0.1			

dry season, only newly emergent efts were found near the stream, probably because their skin had not yet completely developed for a terrestrial lifestyle. A newt (not marked in this study) was found on 14 December 2012 killed on a road two kilometers from the study site, inferring considerable terrestrial movements in this species. Other Asian newts are also known to travel considerable distances on land. Individuals of *T. asperrimus* and *Paramesotriton hongkongensis* moved more than two hundred meters from the breeding stream (Sun et al., 2011; Fu et al*.,* 2013). The North American newt *Notophthalmus viridescens* travels long distances overland (ca. 80 m in one night) during its eft stage (Roe & Grayson, 2008).

During the rainy season, adult newts at the Laos locality inhabited a breeding stream that was surrounded by trees, shrubs, and grasses, similar to that observed in the congeners *T. uyenoi* in Thailand (Khonsue et al., 2010), *T. verrucosus* in India and Bhutan (Kuzmin et al*.,* 1994; Roy & Mushahidunnabi, 2001), and *T. wenxianensis* in Qingchuan, China (Gong & Mu, 2008). In these species, reproduction can occur in a variety of temporary or permanent water bodies that contain vegetation. The breeding season of *T. podichthys* in Laos occurred at the beginning of the rainy season (June–July). This pattern is also seen in other Asian newts. *Tylototriton verrucosus* in India breeds during March–June (Roy & Mushahidunnabi, 2001), *T. wenxianensis* in Qingchuan, China, breeds during April–May. Courtship was not observed in this study, but presumably it occurs under moist grasses in the dry stream during rain, as adult newts were found in the dry grassy stream during and immediately after rain. At our study site, eggs were laid on the moist underside of vegetation in dry stream pools before they filled with water. There, eggs developed and were ready to hatch at the time the stream filled with rain water. *Tylototriton shanjing* at Yunnan Province, China, exhibited terrestrial and aquatic courtship during the early rainy season (May–June), and then females laid eggs one to two days after mating (Jun et al., 2012).

Larvae of *T. podichthys* were found in August living at the stream bottom, and efts emerged onto land by December. In captivity, *T. shanjing* larvae hatched after 15–40 days, metamorphosis began approximately 60 days later, and emergent efts had a total length of 40–45 mm (Ziegler et al, 2008). At the study site, efts grew rapidly, but growth rate decreased after reaching sexual maturity. Our recapture of an eft indicates that *T. podichthys* probably matures after at least two years. Likewise, Kuzmin et al. (1994), Khonsue et al. (2010), and Seglie et al. (2010) found that *T. verrucosus* matured in two to four years.

The diet of *T. podichthys* consisted of terrestrial, but not aquatic, arthropods probably because the newts spent such a brief period in the water. This is in contrast to *T. verrucosus* at Darjeeling, India, that had both aquatic and terrestrial adults and therefore aquatic and terrestrial elements in their diet consisting of mostly amphibian eggs, amphibian larvae, and earthworms (Dasgupta, 1996). Many newts eat the aquatic eggs of their own species (Wood & Goodwin, 1954; Kaplan & Sherman, 1980; Phimmachak et al., 2012; Fu et al., 2013). However, conspecific oophagy was not documented in *T. podichthys* in this study, probably because feeding is limited or absent during the brief period of aquatic courtship and mating.

Many species of Asian newts are threatened with extinction from overexploitation for traditional medicine and the international pet trade (IUCN, 2014). In Laos, the newt *Laotriton laoensis* is highly threatened from overharvesting (Stuart et al., 2006; Phimmachak et al., 2012; Stuart et al., 2014). Local people reported that foreign visitors harvested or purchased *T. podichthys* in 2007 and 2008 in Kham and Phoukhout District, Laos, for pet and medicinal purposes. However, the current primary threat to *T. podichthys* at the study site is habitat loss. Natural habitats there are rapidly being modified for agricultural purposes using slash and burn methods and chemicals (herbicides and fertilisers). The terrestrial period of efts and adults requires intact, humid forest. Consequently, these human activities may result in the extirpation of this newt population in the near future.

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