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Diet composition and neighboring prey community of the Phuping newt (*Tylototriton uyenoi*) in Maesa–Kogma Biosphere Reserve, Chiang Mai Province, northern Thailand

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Abstract. *Dowwiangkan T, Chuaynkern Y, Dumrongrojwattana P, Duengkae P. 2020. Diet composition and neighboring prey community of the Phuping newt (*Tylototriton uyenoi*) in Maesa–Kogma Biosphere Reserve, Chiang Mai Province, northern Thailand. Biodiversitas 21: 4515-4523.* The present work reports the comparison of fecal pellet prey composition of *Tylototriton uyenoi* from Maesa-Kogma Biosphere Reserve (Chiang Mai Province, northern Thailand) based on an analysis of its fecal pellets. Males of *T. uyenoi* consumed more diverse prey than those of females (53 groups of prey versus 14 groups). The analysis reveals a diverse diet of Arthropoda (Insecta, Arachnida, Diplopoda, Chilopoda, and Malacostraca), Mollusca (Gastropoda), Chordata, and conspecific eggs. Partial remnants of plant materials were also found in the pellets. The percentage frequency of prey occurrence was higher in Formicidae (21.34), Lymnaeidae (17.39), unknown snails (10.67), and Blattidae (9.881). The percentage of the relative number of preys was higher in Lymnaeidae (41.02) and Formicidae (17.17). The volumetric prey percentage was higher in Lymnaeidae (30.78), Paradoxosomatidae (19.14), and unknown snails (12.71). The index of relative importance was higher in Lymnaeidae (1,248.62), Formicidae (376.972), unknown snails (188.275), and Paradoxosomatidae (166.433). The percentage of IRI was higher in Lymnaeidae (57.128) and Formicidae (17.248). The Spearman rank coefficient (95% confidence interval test) showed a significant positive correlation ($p<0.05$) between prey volume and number and SVL.

Keywords: Ecology, fecal pellet analysis, prey items, Salamandridae

INTRODUCTION

The Phuping newt *Tylototriton uyenoi*, also known as the Asian newt, is the urodele amphibian of the family Salamandridae knew from Thailand (Frost 2020). It was described as a new species in 2013 (Nishikawa et al. 2013). Distribution of the genus *Tylototriton* ranges from the high elevations of the eastern Himalayas to central China and mainland Southeast Asia (Shen et al. 2012; Qian et al. 2017). This includes the lower montane evergreen forest in the Maesa–Kogma Biosphere Reserve in Chiang Mai Province, Thailand. Major populations of *T. uyenoi* can be found in cold climate mountains at elevations of 1,000 m above sea level or more (Pomchote et al. 2008; Nishikawa et al. 2013; Hernandez 2016; Hernandez 2017; Hernandez et al. 2019). The newts live in clearwater streams along the least disturbed mountains and their presence can indicate the health of local montane forest ecosystems (Marod and Kutintara 2009). In addition, they transfer energy from aquatic to land habitats when they metamorphose into a terrestrial stage (Duellman and Trueb 1994; Le et al. 2018). The Phuping newt is currently threatened by extensive deforestation for agricultural activity, shifting cultivation, pollution from human settlement, and overharvesting for the international pet trade (Pattanavibool and Dearden 2002; Chuaynkern and Duengkae 2014; Rowley et al. 2016). Climate change also presents problems in tropical areas as increased temperature could decrease newt populations by changing their life cycle and breeding sites (Bickford et al. 2010). Accordingly, the conservation status of *Tylototriton* populations in Thailand was re-evaluated and is currently listed as a protected by Thai law: The Wild Animal Reservation and Protection Act B.E. 2535 due to taxonomic change (Ministry of Natural Resources and Environmental 2017).

Tylototriton foraging and diet were reported for *Tylototriton himalayanus* (formerly *Tylototriton verrucosus*) in Indian Darjeeling (Kuzmin et al. 1994; Dusgupta 1996) and Manipur state (Devi 2005), India (Devi 2005), as well as for *Tylototriton podichthys* in Xiengkhouang Province, Laos (Phimmachak et al. 2015). According to Pomchote et al. (2008), larvae of *T. verrucosus* feed mainly on small crustaceans (the water fleas *Moina macrocapa*) and also showed cannibalistic behaviors among larval siblings of different sizes. The adult newts consume small invertebrates (bloodworms *Chironomus* sp., mealworms *Tenebrio molitor*, crickets *Acheta* sp.) and vertebrates. The *Tytlototriton* newt's natural feeding and dietary selection patterns in Thailand are poorly known. Based on analysis of fecal pellets, Ponpituk (2014) found that adults *T. uyenoi* in Doi Suthep–Pui National Park (Chiang Mai Province, Thailand) consumed diverse arthropods (Arachnida, Diplopoda, Insecta) and mollusks (Bivalvia). Dowwiangkan et al. (2016) found that adults *T. uyenoi* from Chiang Mai Province (the Royal Agricultural Station Ang Khang and Maesa–Kogma Biosphere Reserve) also consumed arthropods (classes Arachnida, Diplopoda, Insecta) and mollusks (class Bivalvia). As the diet ecology information of the newts in their natural habitat is still far from fully known, our study aims to investigate the food items based on the fecal pellet analysis and food selection of *T. uyenoi* from Maesa–Kogma Biosphere Reserve, northern Thailand. The results of this study add information on dietary of the species and can improve understanding about *Tylototriton* newts feeding ecology and the role that they play in their ecosystems.

MATERIALS AND METHODS

Study area

Maesa–Kogma Biosphere Reserve (Figure 1) is located in Chiang Mai Province, northern Thailand. The area occupies 57,366 hectares, which is mostly part of Doi Suthep–Pui National Park. Field surveys were performed at a 16-hectare (160,000 m^2) permanent plot (Figure 1B) at 1,332 m above sea level in the Maesa–Kogma Biosphere Reserve at the coordinates of 18°48'45.7" N, 98°54'7.7" E. Vegetation type is lower montane forest and the area is the type locality of *T. uyenoi* (Nishikawa et al. 2013). Research and specimen collection were performed under the permission of the Department of National Parks, Wildlife and Plant Conservation (Permission number DNP 0907.4/9819).

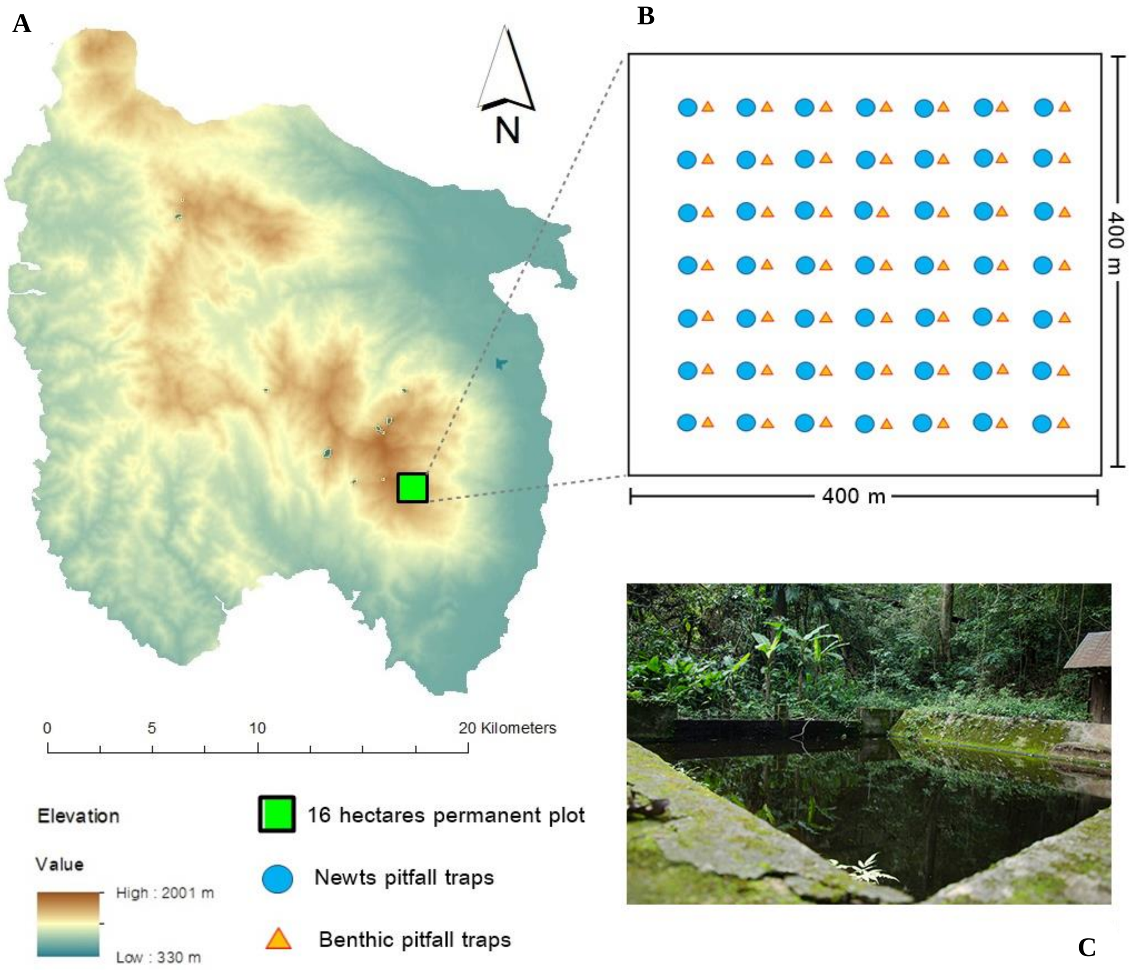

Figure 1. Study site, Maesa–Kogma Biosphere Reserve, Chiang Mai Province, Thailand

Sampling and fecal pellet collection

For trapping *T. uyenoi*, we used 49 plastic tanks 30 cm in diameter and 40 cm deep-set as pitfall traps. These traps were systematically embedded at 50-m intervals throughout the 16-hectare permanent plot (Figure 1.B). Sampling was conducted monthly (3 nights per month) from March 2017 through September 2018. Captured newts were placed in 16x26 inch transparent plastic bags (one per newt) and kept overnight. On the following morning, we collected fecal pellets from each plastic bag and preserved them in 70% ethanol for identifying food items in the laboratory (Chuaynkern et al. 2009; Thongproh et al. 2019). After collecting feces, we marked the newts by injecting colored, visible implant elastomer (VIE) tags (Northwest Marine Technology Inc., Shaw Island, WA) below the skin with a 0.3-ml insulin syringe. We marked the newts at four points 5-10 mm behind the cloaca according to Lunghi et al. (2014) and Dowwiangkan et al. (2018). The captured newts were weighed using digital scales. We measured six morphological characteristics using an analog vernier caliper. Measurements were as follow: SVL (snout to vent length) measured from tip of snout to anterior tip of the vent, TL (tail length) measured from anterior tip of the vent to the tip of the tail, TTL (total length) measured from tip of snout to tip of the tail, MW (mouth width) measured distance between corner of the mouth, HW (head width) measured between position of maximum head width. We identified the sex of the newts by the cloacal shape. Finally, the newts were released to their natural habitat.

For investigating the availability of terrestrial foods in their natural habitat, we used the benthic pitfall trapping technique (Woodcock 2005). The smaller benthic pitfall traps were 49 cylindrical plastic containers 10 cm in diameter and 15 cm deep-set alongside the newt pitfall traps. Each benthic trap was filled with 50% ethanol at a volume 1/3 of the depth of the trap. We also investigated aquatic food in artificial pond located in the16-hectare (160,000 m^2) permanent plot (Figure 1.C). by using 30x30 cm iron net boxes (mesh size 1x1 mm) as in Dusgupta (1996). A total of 16 boxes were placed on the bottom of the pond near pond edges and in the middle of the pond. All captured aquatic animals were preserved in 70% ethanol.

Food item analysis

Fecal pellet contents and terrestrial and aquatic communities were identified using a stereomicroscope at the Faculty of Forestry at Kasetsart University, Bangkok. Prey fragments found in pellets and terrestrial and aquatic organisms found in traps were identified via comparison with relevant taxonomic references (Chuensri 1974; Monzon et al. 1993; Naiyanetr 1997; Triplehorn and Johnson 2005; Inmadon et al. 2011; Ponpituk 2014). Items were also photographed and measured using a digital caliper.

Index of Relative Importance (IRI) for each prey type in newt diet was calculated 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 (Pinkas et al. 1971). We also converted the IRI as percentage of the total index (followed Oliveira et al. 2007), %IRI = (IRR / Σ IRI) x 100. Correlation between volume of prey consumed and body size was tested using Spearman's rank correlation. The mean with 95% confidence intervals was used to evaluate the relationship between body size (MW, HW, SVL, TTL, weight) and prey volume in each sex (Griffiths 1986).

RESULTS AND DISCUSSION

Results

We collected 253 fecal pellets from 295 trapped individuals of *T. uyenoi* (209 males and 86 females) at 440 capture times. Food items were found in 220 pellets but not in the remaining 33 pellets. Based on analysis of the 220 fecal pellets containing identifiable food, we identified 626 individual food items that were distributed in three phyla: Arthropoda, Mollusca, and eggs of *T. uyenoi* (Table 1). Food items could be divided further into 57 taxonomic categories, with most categories coming from taxa that occupied the terrestrial habitat (91.22%) and only 8.77% of aquatic taxa. Males of *T. uyenoi* consumed more diverse prey than those of females (53 groups of prey versus 14 groups). The males consumed mainly on arthropods (43 groups), mollusks (9 groups), and eggs of *T. uyenoi*. Percentage frequency of occurrence (%F) was higher in Formicidae (19.763) and Lymnaeidae (17.391). Percentage relative number of individuals of prey (%N) was higher in Lymnaeidae (41.018) and Formicidae (16.375). Percentage volumetric (%V) was higher in Ixodidae (0.234) and Histeridae (0.096). The index of relative importance (IRI) was higher in Paradoxosomatidae (2,073.704) and Potamidae (2,012.536). The percentage of IRI (%IRI) was higher in Paradoxosomatidae (94.88) and Potamidae (92.08). Like the males, the females consumed mainly on arthropods (11 groups) and mollusks (3 groups). Percentage frequency of occurrence was higher in Paradoxosomatidae (1.976) and Pseudoscorpiones (1.86) and unknown snails (1.186). Percentage relative number of individuals of prey was higher in Paradoxosomatidae (0.795) and unknown snails (0.795) and Isoptera (0.636). Percentage volumetric was higher in Paradoxosomatidae (4.217) and unknown snails (1.482). The index of relative importance (IRI) was higher in Paradoxosomatidae (2.536), Potamidae (2.481), and unknown snails (1.92). The percentage of IRI was higher in Paradoxosomatidae (0.116) and Potamidae (0.113).

In both sexes, *T. uyenoi* were found throughout the year (except in March 2018 that was not surveyed), most often during the rainy season and least often in the dry season. In 2017, *T. uyenoi* were captured in all months from March through December (Figure 2). Similarly, most of the fecal pellets were obtained during the rainy season, especially in May through August. For 2018, the number of captured newts and collected fecal pellets were similar to those of the previous year.

Analysis of fecal pellet contents of males and females showed that *T. uyenoi* consumed diverse arthropods (46 groups), mollusks (10 groups), and eggs of *T. uyenoi*. The newts ate five classes of Arthropoda: Insecta (12 orders), Arachnida (4 orders), Diplopoda (2 orders), Chilopoda (1 order), and Malacostraca (1 order). For Mollusca, *T. uyenoi* ate 1 class, Gastropoda (3 orders). In Chordata, *T. uyenoi* ate amphibian eggs. Prey type frequency was higher in Arthropoda (70.75%) and Mollusca (Gastropoda: 36.36%). However, Gastropoda represented the highest proportion in number and volume of food items. We also found plant materials (*e.g.*, seed, leaf fragments, mosses) and pebbles, but those were excluded from our analysis. Percentage frequency of occurrence was higher in Formicidae (21.34), Lymnaeidae (17.39), unknown snails (10.67), and Blattidae (9.881). Percentage relative number of individuals of prey was higher in Lymnaeidae (41.02) and Formicidae (17.17). Percentage volumetric was higher in Lymnaeidae (30.78), Paradoxosomatidae (19.14), and unknown snails (12.71). The index of relative importance was higher in Lymnaeidae (1,248.62), Formicidae (376.972), unknown snails (188.275), and Paradoxosomatidae (166.433). The percentage of IRI was higher in Lymnaeidae (57.128) and Formicidae (17.248).

The Spearman rank coefficient (95% confidence interval test) showed a significant positive correlation (r_s [rho] = -0.1270, p = 0.0443 (p<0.05)) between prey volume and SVL (Figure 3). The prey volume and mouth (r_s (rho) = -0.0482, p = 0.4454), prey volume and weight (r_s [rho] = -0.0711, p = 0.2595), and prey volume and total length (r_s [rho] = -0.0081, p = 0.1979) had negative correlation (p<0.05).

Number of prey individuals and SVL had a significant positive correlation (r_s (rho) = -0.1563, p = 0.0128, p<0.05) while as number of prey volume and mouth (r_s (rho) = -0.0713, p = 0.2583), number of prey volume and weight (r_s (rho) = -0.0037, p = 0.9536), and number of prey volume and total length (rs (rho) = -0.1082, p = 0.0858) had negative correlation (p<0.05) (Figure 4).

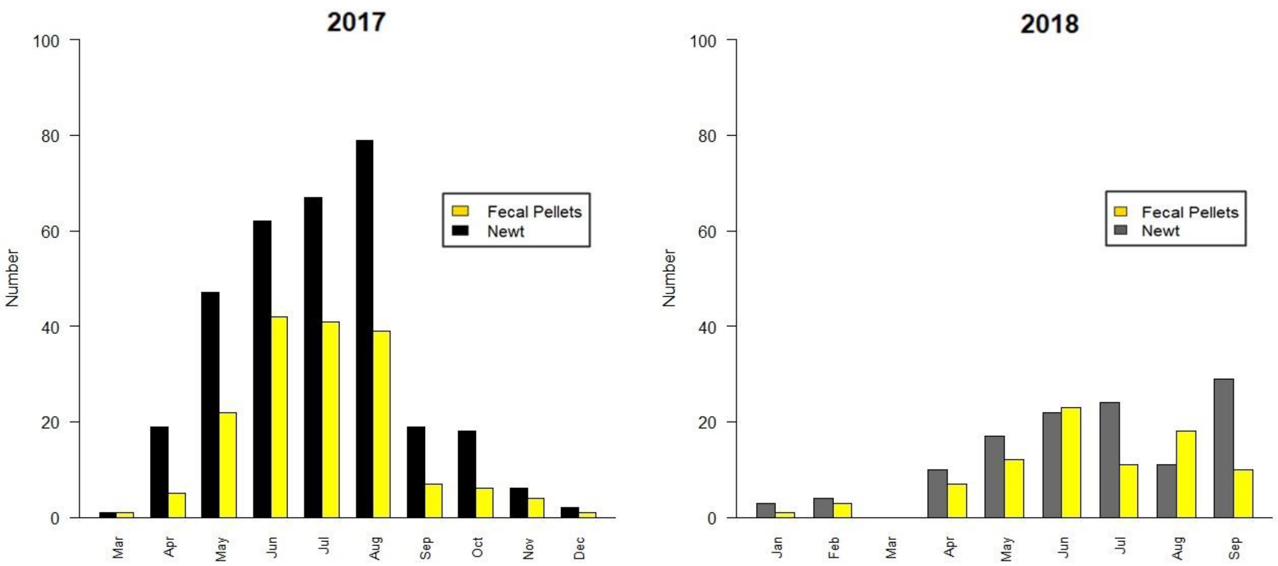

Figure 2. Captured newts and fecal pellets collected each month during the study period, March 2017 through September 2018. Note that March was not surveyed in 2018.

Table 1. Diet composition of *Tylototriton uyenoi* collected from Maesa–Kogma Biosphere Reserve, Chiang Mai Province, Thailand

Phylum	Class	Prey items	Nich	%F	%N	%V	IRI	%IRI
Arthropoda	Insecta	Order Archeognatha	Ter	0.345	0.159	0.002	0.064	0.003
		Order Odonata						
		Suborder Anisoptera	Aq	1.976	0.795	3.661	8.807	0.403
		Suborder Zygoptera	Aq	3.162	1.272	0.346	5.116	0.234
		***Nymph	Aq	0.395	0.159	0.053	0.084	0.004
		Order Orthoptera						
		Family Gryllidae	Ter	3.162	1.272	1.453	8.618	0.394
		Family Acrididae	Ter	0.791	0.318	0.216	0.422	0.019
		Family Tettigoniidae	Ter	0.791	0.318	0.340	0.520	0.024
		Order Blattodea						
		Family Corydiidae	Ter	0.791	0.318	0.535	0.675	0.031
		Family Blattidae	Ter	9.881	3.975	2.034	59.370	2.716

		Order Isoptera	Ter	2.767	2.226	0.127	6.510	0.298
		Order Dermaptera						
		Family Cacinophoridae	Ter	5.138	2.067	6.573	44.396	2.031
		Family Forficulidae	Ter	0.395	0.159	0.557	0.283	0.013
		Order Hemiptera						
		Family Lygaeidae	Ter	0.395	0.159	0.604	0.301	0.014
		***Nymph	Ter	0.395	0.159	0.005	0.065	0.003
		Order Homoptera						
		Family Delphacidae	Ter	0.395	0.159	0.155	0.124	0.006
		Order Coleoptera						
		Family Coccinellidae	Ter	0.395	0.159	0.194	0.140	0.006
		Family Curculionidae	Ter	3.162	1.431	1.504	9.279	0.425
		Family Attelabidae	Ter	0.395	0.159	0.157	0.125	0.006
		Family Staphylinidae	Ter	0.395	0.159	0.064	0.088	0.004
		Family Elateridae	Ter	1.976	0.795	0.879	3.308	0.151
		Family Buprestidae	Ter	0.791	0.318	0.107	0.336	0.015
		Family Tenebrionidae	Ter	1.186	0.477	0.344	0.974	0.045
		Family Cerambycidae	Ter	0.395	0.159	0.374	0.211	0.010
		Family Endomychidae	Ter	0.395	0.159	0.220	0.150	0.007
		Family Histeridae	Ter	0.395	0.477	0.096	0.227	0.010
		Family Trogidae	Ter	0.395	0.159	0.054	0.084	0.004
		Family Unknown	Ter	0.395	0.159	0.197	0.141	0.006
		***Larvae	Ter	3.557	1.431	1.830	11.601	0.531
		Order Lepidoptera						
		Family Psychidae	Ter	1.581	1.749	0.464	3.498	0.160
		***Pupae	Ter	0.395	0.159	0.385	0.215	0.010
		**Larvae	Ter	0.791	0.318	0.171	0.387	0.018
		Order Diptera	Ter	0.791	0.318	0.152	0.372	0.017
		Family Tachinidae	Ter	0.395	0.159	0.084	0.096	0.004
		Family Tephritidae	Ter	0.791	0.318	0.105	0.335	0.015
		***Larvae	Ter	0.791	0.318	0.152	0.372	0.017
		Order Hymenoptera						
		Family Formicidae	Ter	21.340	17.170	0.492	376.972	17.248
		Family Vespidae	Ter	0.395	0.159	0.037	0.078	0.004
	Arachnida	Order Araneae						
		Family Salticidae	Ter	2.372	1.113	1.732	6.747	0.309
		Family Sparassidae	Ter	0.791	0.318	0.368	0.542	0.025
		Order Opiliones						
		Family Sclerosomatidae	Ter	0.791	0.318	0.562	0.696	0.032
		Order Pseudoscorpiones	Ter	1.976	0.954	0.325	2.527	0.116
		Order Scopiones						
		Family Scorpionidae	Ter	0.395	0.159	0.234	0.155	0.007
	Diplopoda	Order Julida	Ter	0.791	0.318	0.856	0.928	0.042
		Order Polydesmida						
		Family Paradoxosomatidae	Ter	7.51	3.021	19.14	166.433	7.615
	Chilopoda	Order Scolopendromorpha	Ter	1.581	0.636	0.642	2.021	0.092
	Malacostraca	Order Decapoda						
		Family Potamoidea	Ter	2.372	0.954	5.108	14.376	0.658
Mollusca	Gastropoda	Order Mesogastropoda						
		Family Lymnaeidae	Aq	17.390	41.020	30.780	1248.620	57.128
		Family Cyclophoridae	Ter	0.791	0.318	0.044	0.286	0.013
		Order Stylommatophora						
		Family Camaenidae	Ter	0.791	0.477	0.368	0.668	0.031
		Family Clausiliidae	Ter	1.186	0.477	0.413	1.055	0.048
		Family Subulinidae	Ter	1.581	0.795	0.649	2.283	0.104
		Family Helicarionidae	Ter	1.976	0.795	1.338	4.216	0.193
		Family Pyramidulidae	Aq	1.581	0.795	0.120	1.446	0.066
		Order Architaenioglossa	Ter	0.791	0.477	0.368	0.668	0.031
		Diplommatinidae	Ter	0.791	0.318	0.019	0.267	0.012
		Unknown snails	Ter	10.67	4.928	12.710	188.275	8.614
Chordata	Amphibia	Order Urodela						
		Family Salamandridae [Eggs]	Ter	0.395	0.318	0.014	0.131	0.006

Note: Aq: aquatic, Ter: terrestrial

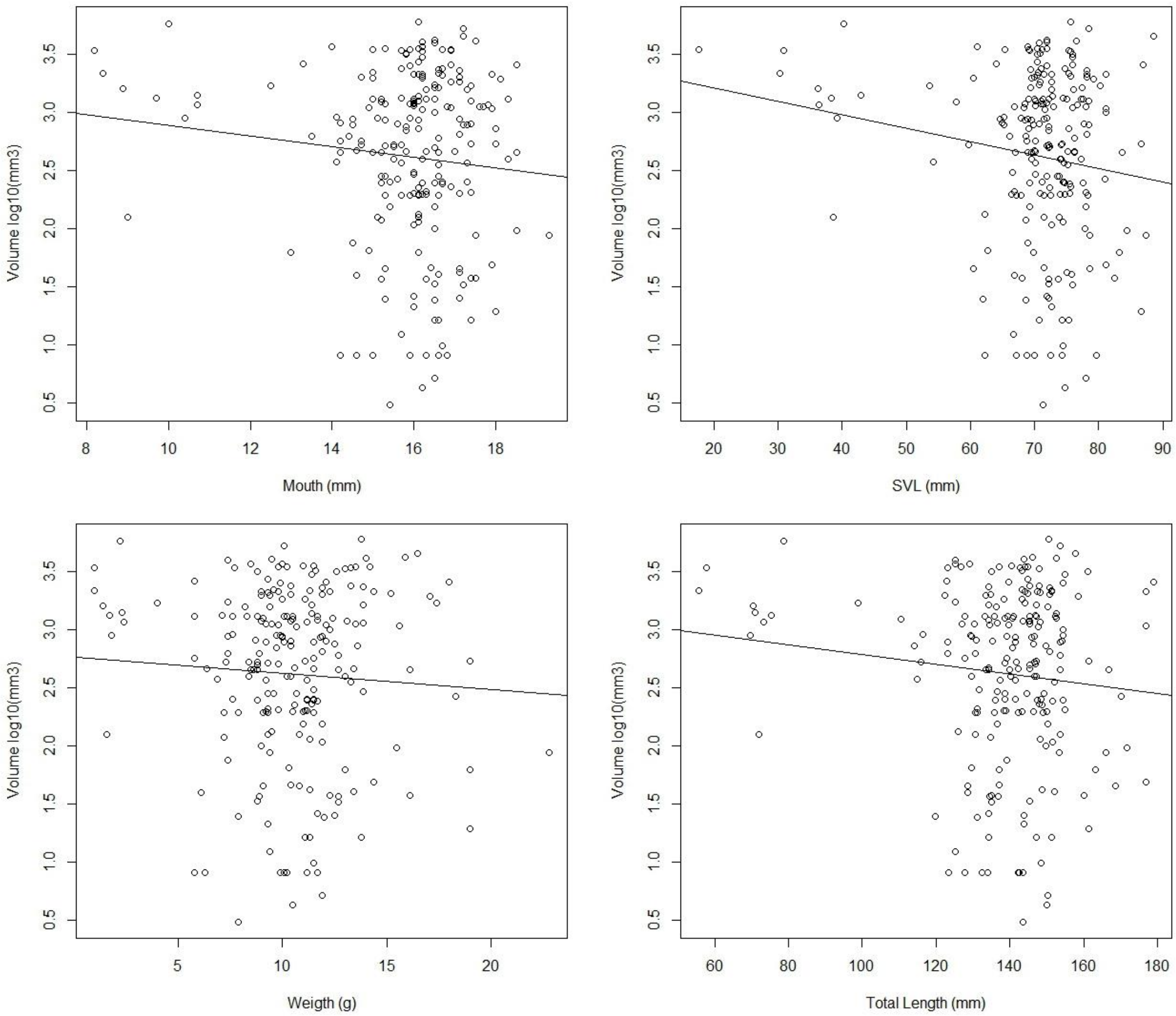

Figure 3. The Spearman rank coefficient (95% confidence interval test) comparison of prey volume versus mouth width, SVL, body weight and total length. A. prey volume versus mouth, B. prey volume versus SVL, C. prey volume versus weight, D. prey volume versus total length. Prey volume and SVL had a significant negative correlation ($p<0.05$)

Discussion

Dowwiangkan et al. (2016) analyzed 67 fecal pellets obtained from 102 individuals of *T. uyenoi* which were collected in two periods (January-December 2013 and April-September 2015) from the Royal Agricultural Station Ang Khang (55 fecal pellets from 81 individuals) and Maesa-Kogma Biosphere Reserve (12 fecal pellets from 21 individuals), Chiang Mai Province, northern Thailand. The diets consisted of two phyla: Arthropoda (classes Arachnida, Diplopoda, Insecta) and Mollusca (class Bivalvia). Unlike Dowwiangkan et al. (2016), we did not observe four families of the class Insecta in fecal pellets: Carabidae (order Coleoptera), Chelisochidae (order Dermaptera), and Gryllacrididae (order Orthoptera). Our findings also included one more phylum, Chordata (class Amphibia: Urodela/Salamandridae). We found parts of newts' eggs in the fecal pellets of the male. Conspecific oophagy is also seen in *T. verrucosus* from India (Kuzmin et al. 1994; Dasgupta 1996; Lucy et al. 2014) and other newts (see e.g., Wood and Goodwin 1954; Kaplan and Sherman 1980). Dasgupta (1996) and Lucy et al. (2014) only found newt eggs in the stomach contents of females and denoted that the Himalayan newt oophagy is sex-specific. Sex-specific oophagy has also been reported in other newt species such as *Taricha torosa* (Marshall et al. 1990). However, Phimmachak et al. (2015) did not observe conspecific oophagy in *T. podichthys* from Laos. In our work, *T. uyenoi* shows the oophagy in the adult male as part of egg material was found in the fecal pellet. Contrast to Dasgupta (1996) that found only female *T. verrucosus* (Darjeeling, India) cannibalized eggs. In our study, males of *T. uyenoi* consumed more diverse prey than those of females. During breeding period, the diets of adult males and females *T. verrucosus* comprised both aquatic and terrestrial elements. The males consumed more diets than females during the post-breeding period while the female consumed more during the breeding period. However, Phimmachak et al. (2015) found the diet composition, number of prey consumed, and volume of prey consumed did not differ among adult males and females.

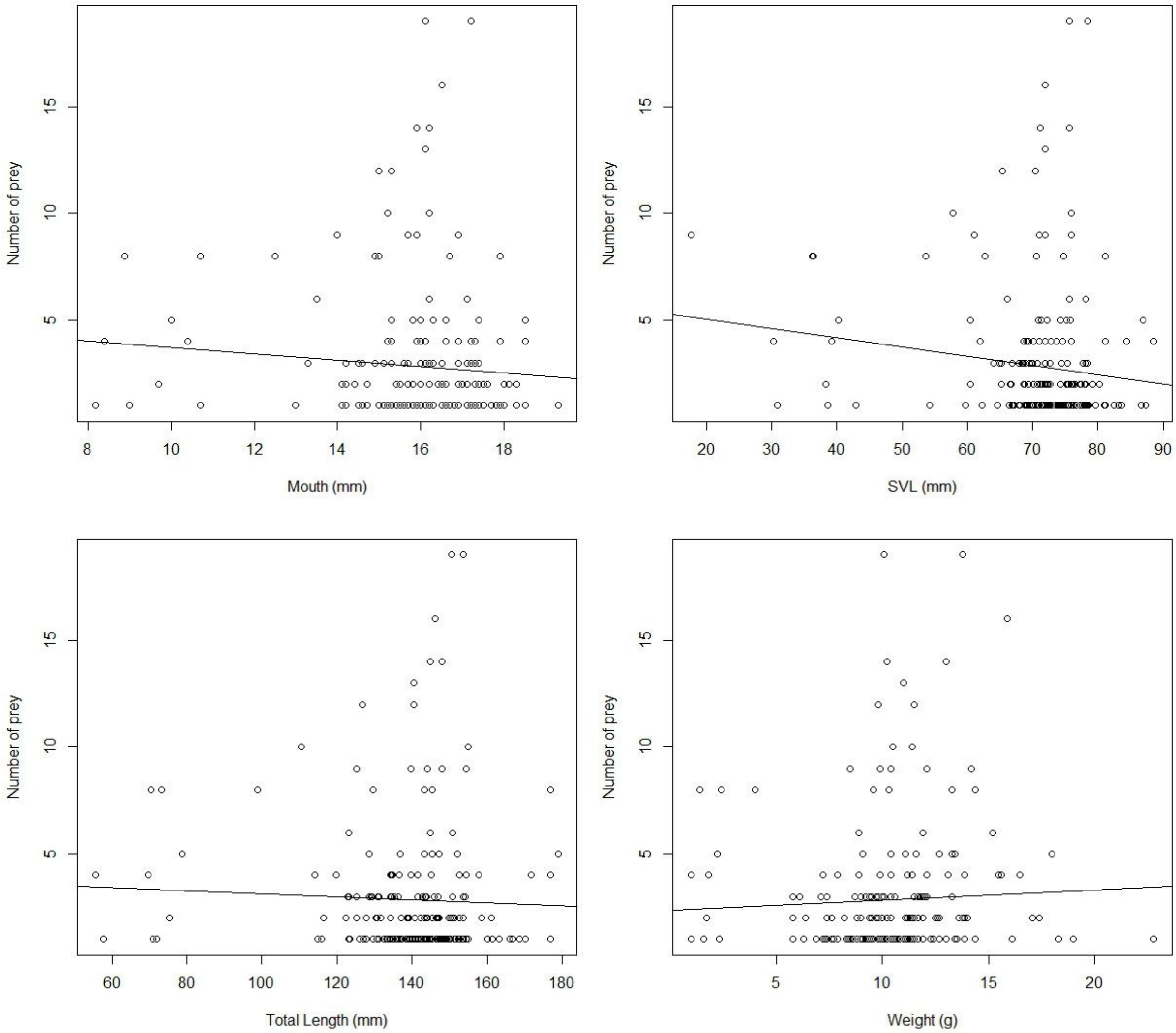

Figure 4. The Spearman rank coefficient (95% confidence interval test) comparison of number of prey individuals versus mouth, SVL, weight, and total length. A. prey volume versus mouth, B. prey volume versus SVL, C. prey volume versus weight, D. prey volume versus total length. Number of prey individuals and SVL had a significant negative correlation (p<0.05).

The Phuping newt appears to be a generalist predator rather than a specialist. The most important food was arthropods as similar to those of previous works investigated on amphibians from Thailand (see *e.g.*, Chuaynkern et al. 2009; Ponpituk 2014; Thongproh et al. 2019). There are five most important groups represented in the diet of the Phuping newt: Formicidae (ants), Lymnaeidae (pond snails), unknown snails, Blattidae (cockroach), and Paradoxosomatidae (flat-backed millipedes). However, there are many ways to assess animal diets. With the observational method (*e.g.*, Kaplan and Sherman 1980) the researcher directly observes the food eaten by the animal. With the fecal pellet analysis method (*e.g.*, Chuaynkern et al. 2009; Thongproh et al. 2019), feces are collected from the animals and the remaining part of food from digestion is identified. The stomach-flushing method (*e.g.*, Dasgupta 1996; Lucy et al. 2014; Wu et al. 2007; Phimmachak et al. 2015), collects food by flushing their stomachs. With the stomach-dissecting method (Wu et al. 2007), the animal is killed and its stomach is cut and take off. As *T. uyenoi* is listed as protected animal by Thai law (Ministry of Natural Resources and Environmental 2017) and investigation on dietary is required numerous individuals, therefore the fecal pellet analysis is appropriated and selected for our study method. However, the observational method and fecal pellet analysis may be received the exactly consumed preys because the methods are difficult and the prey items might be completely digested or unrecognizable. Our previous work based on fecal pellet analysis evidenced good recognizable prey items (Chuaynkern et al. 2009; Ponpituk 2014; Dowwiangkan et al. 2016).

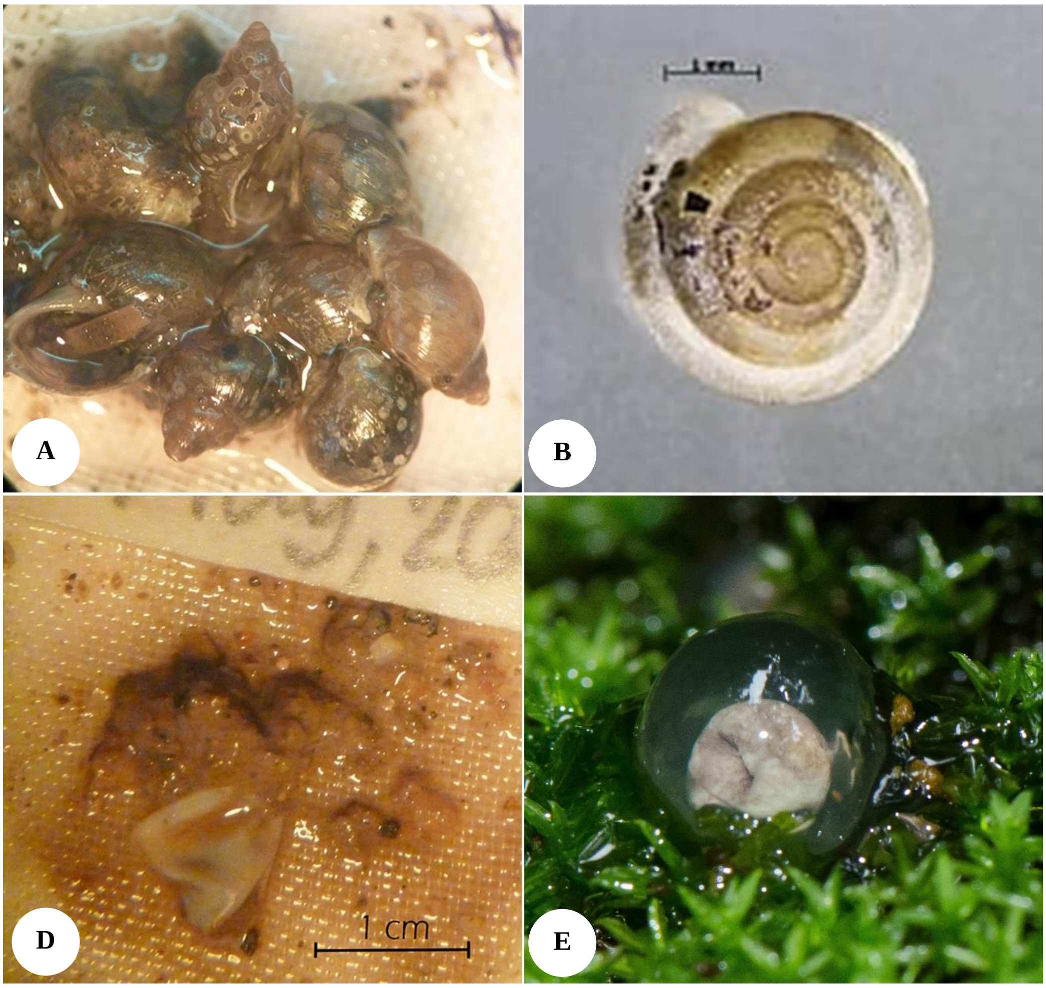

Figure 5. Selective food items observed in the Uyenoi' newt. A. Snail species 1, B. Snail species 2, C. Unhatched egg of the Phuping newt obtained from the fecal pellet, D. Unhatched egg of the Phuping newt from oviposition

However, the main problem with the fecal pellet analysis might be completely digested prey. Dowwiangkan et al. (2016) and the present work did not find earthworms in the fecal pellets of *T. uyenoi* whereas earthworms were found in the diet of other newts based on the flushing technique: *T. verrucosus* (Dasgupta 1996; Lucy et al. 2014) or *T. podichthys* (Phimmachak et al. 2015). Therefore, *T. uyenoi* might eat earthworms, but the prey may have been completely digested before production of a fecal pellet and could not be observed. According to Solé et al. (2005), the stomach-flushing method is useful for dietary analyses of protected species or populations under long term study as it was claimed that the method does not require killing the animals to obtain dietary data. However, Joly (1987) showed that treatment can sometimes cause mortality.

The ecological niche of the prey categories found in the pellet indicates that the Phuping newts search for their food mainly on the ground (91.22%) rather than in water (8.77%). This is not congruent with *T. verrucosus* from Darjeeling Himalayas (India) in which these ratios are equal (Dasgupta 1996). However, the diet of studied newts consistently comprised both terrestrial and aquatic prey as presented in Lucy et al. (2014), Phimmachak et al. (2015), and Dowwiangkan et al. (2016).

Today, we are in a worldwide situation in which many animals face declining numbers. Therefore, killing newts for dietary study or other disciplines is even less justified. Improved techniques are recommended to avoid killing of numerous animals for nutritional studies, in particular, because of the worldwide threat to amphibian populations.

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