Malayan Nature Journal 2022, 74(2), 337-346

Prey-handling in the Bornean Keeled Pit-viper *Tropidolaemus subannulatus*

*VERONICA ANAK MARTIN and INDRANEIL DAS

Abstract : The plasticity of feeding behaviour of predators is strongly influenced by foraging mode, depending on whether they are active foragers, sit-and-wait predators or opportunist feeders. In this study, we conducted ex-situ feeding experiments on the Bornean Keeled Pit-viper, *Tropidolaemus subannulatus*, a lowland rainforest species distributed on Borneo, Sulawesi and the Philippines. Observations were based on four wild-collected females maintained under laboratory conditions. A total of eight common predatory behaviours were observed that can be classified into three discrete phases, namely, pre-capture, feeding, and post-feeding phases, during experiments with new-born and live young *Rattus norvegicus*. In the pre-capture phase, which is temporally the shortest, there were head shifts, eye fixation and head movement towards prey. During the long feeding phase, actions involved strikes, awaiting to ensure prey death, and swallowing of prey. Post-feeding phase is a process of muscular recovery, followed by high-rate of tongue flicks, that can last for up to 15 min. Understanding foraging and prey-handling behaviour has the potential to provide deeper understanding on evolutionary fitness, as well as the biotic and abiotic factors which interacts with the concerned species.

Keywords: snake, ethogram, feeding, prey predator, behaviour.

INTRODUCTION

Prey immobilisation options available for snakes that consume large prey are constrained by their lack of limbs, and restricted largely to constriction or envenomation. Striking is a distinctive technique of defence as well as a predatory mechanism (Lillywhite 2014). A predatory strike is apparently calculated and carefully executed, a successful strike requiring the capability to cover spatial distance between the predator and its target, ensuring the latter has no time to respond to the strike, and be accurately make physical contact with the target (Young 2010). Snakes can strike at speeds beyond the tracking capacity of the human eye, and it has been reported that a snake lunge can take under half a second, from resting position, erection of fangs, injection of venom and return to initial stance (Kardong 1986, Kardong and Bels 1998, LaDuc 2002).

Among venomous species, envenomation strategy tends to be different, depending on the nature of prey. Larger prey tends to be envenomated and immediately released, since large prey may be dangerous and can actively struggle. On the other hand, snakes typically maintain a grasp on smaller prey types, and continue to hold them in the mouth firmly prior to swallowing, which takes place after the prey ceases to struggle (Lillywhite 2014). Snakes typically transport their prey via asynchronous ratcheting movements of their upper jaws in which the jaws from left or right side of the head, alternately move over the prey (Gans 1961, Cundall 1987, Kley and Brainerd 1999), suggesting that the lower jaws have little direct role in prey transportation, but act as a control of prey position in the mouth and to press it against teeth of the overlying palatine and pterygoid bones.

[1]Institute of Biodiversity and Environmental Conservation,Universiti Malaysia Sarawak,
94300, Kota Samarahan, Sarawak, Malaysia
***Corresponding email:** vero35nica@gmail.com

Tropidolaemus subannulatus, the Bornean Green Keeled Pit-viper, is a lowland rainforest species distributed across Borneo, as well as several other smaller Sundaic islands, Sulawesi and the southern Philippine islands. It is known to feed on birds and small mammals (Stuebing *et al.* 2014), although further details regarding its predatory behaviour are lacking in the literature. The obvious variation displayed by the species is in colouration among the females. Some appear yellow-green, with blueish crossbands, while some other turn blue-black, with small yellowish green dots arranged in a crossband pattern.

Tropidolaemus subannulatus, as with other members of its subfamily (Crotalinae), are thought to identify prey through specialized infrared (thermal) receptors, apart from being aided by other senses, such as vision and chemoreceptors (de Cock Buning 1983, Shine and Sun 2002). Prey size tends to increase with the predator body size (Brandl et al. 1994; Costa *et al*. 2008). A study by Shine and Sun (2002) on the Shedao Island Pit-viper, *Gloydius shedaoensis* demonstrates that prey size increases with snake body size and suggest that small snakes may be limited to feeding on small birds either due to a physical inability to ingest larger prey related to gape-limitation, a preference for smaller birds, or due to ecological or behavioural factors that reduce encounter rate with larger birds. Given the poor knowledge of the life history of the target species, it was thought that understanding details of its feeding behaviour is critical for comprehending its ecological requirements.

MATERIALS & METHODS

Pit-vipers were collected from Kubah National Park (N 01°36.683' E 110°11.725') from lowland forest habitat. The forest vegetation primarily consists of mixed dipterocarp, with small areas of scrub forest and patches of Kerangas or Bornean heath (Hazebroek and Morshidi 2000). A lab setting was established, where a transparent glass tank measuring 90 cm x 45 cm x 45 cm was used. A 5 cm x 5 cm printed grid was placed on a frontal plane and at the bottom of the tank, to facilitate the estimation of distance and for scale. The mean ambient temperature at the lab was 26°C.

A video recorder Sony™ HDR-XR350, set for 24 Mbps shooting rate, was mounted onto a tripod (Manfrotto 055YPron Pro Tripod, with Pro Ball Head 468 MH054MO-Q5) and placed in front of the setting to record all behavioural activities as shown in Figure 1. Live prey used were newborn pinkies weighing between 2.0 to 2.4 gm and fuzzies weighing between 10 to 15 gm. of *Rattus norvegicus*, which were placed 20 cm away from the head of the snake. While some pinkies remained at the spot they were introduced, some pinkies were capable of waddling around the tank until they went close enough to receive a strike by the snakes. All results were captured with high-speed digital videography. In order to analyse their behaviour, snakes acquired from the field were placed in the tank for acclimatisation. The snakes were not fed at least one week prior to the experiment. A total of 15 feeding attempts/sequences were recorded for the feeding behaviour analysis, with four successful feeding experiments. A total of six adult females, three males and six juveniles were used for these feeding behaviour trials. With adult or near adult females displaying the morphological features mentioned; males and juveniles were sexed to check for the presence of hemipenes.

Of these, four females were successfully fed. Video records of their predatory behaviour were compared, and the common behavioural steps were documented. The MTS video files of feeding behaviour captured were analysed with Lightworks (version 2021.1). An ethogram was constructed to interpret predatory behaviour. Distinctive behavioural acts, such as tongue flicks before the strike, and of mandibular retractions and gapes after feeding, were recorded. At the end of the experiment, each individual was released at the respective point of capture.

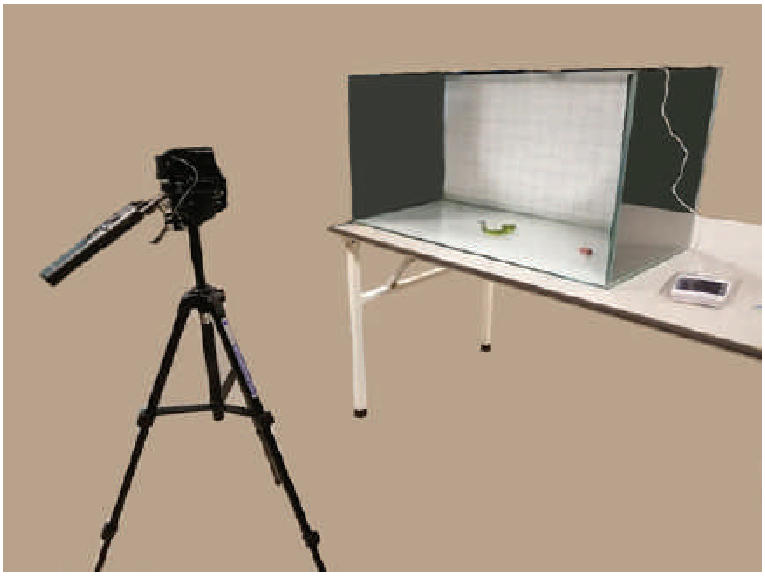

Figure 1. Lab setting on prey handling experiment. A SonyTM HDR-XR350 video recorder, mounted onto a tripod was placed in front of the setting to record all behavioural activities.

RESULTS

Snake Response and Behaviour

In the 15 experiments, seven snakes shifted their heads towards the direction of prey at the moment of introduction of the prey to the tank. Only four (of 15; 26%) individuals successfully completed the process of striking, killing and ingestion, while the rest appeared uninterested and ignored the prey, with no kill nor envenomation or strike behaviour observed. The snakes that failed to show a response to the prey remained motionless, without attempting to investigate the prey being introduced. One individual that approached the prey offered recorded the tongue flick behaviour, but returned to its original perch without further action relevant to predation.

Little variation in behaviour among snake individuals that took an interest in and subsequently ingested the prey were recorded. Three subsequent phases were identified in the predatory behaviour leading to a successful feed, as shown in Table 1. The first is the pre-capture phase, which started with the snake noticing the prey. The second phase began with a strike at prey and continued until entire prey ingestion took place. The final phase is the post-feeding phase, starting after prey has been completely swallowed, followed by jaw adjustment (or 'the yawn') after the feeding event. Thereafter, snakes typically remained motionless (three of four individuals), except for one which became active and moved around the tank in an apparent bid to escape.

Pre-capture Phase

Pre-capture phase involved the snake detecting and approaching the prey. Detection is here defined as when the snake clearly noticed the prey, with its head turned towards it. When the prey was transferred into the tank for the experiment, the state of detection is thought to occur when the respective snake shifted the head towards the direction of the prey and seemingly observed the target to be. This action is interpreted as the snake acknowledging and taking an interest towards the prey. The next step being approach, the snake slowly aligning its head towards the prey.

Feeding Phase

Three behavioural displays were recorded during this phase, namely prey capture, prey evaluation and prey ingestion. Prey capture is the process whereby the snakes move their heads toward the prey, begin to strike once its head is approximately 10 cm away from the prey and presumably proceed to envenomate the victim. Striking began as the snake's head launched forward with the mouth wide open simultaneously. The prey is either released immediately upon presumed envenomation or continued to be retained in the jaws. The next subphase is prey evaluation, where the snake presumably waits, presumably to make sure that the prey is dead, before starting the process of ingestion. During this subphase, snakes tend to stay motionless. The final act is that of swallowing, where the snake starts to transport the prey into the oesophagus. In Tropidolaemus subannulatus, the swallowing mechanism displayed is via asynchronous ratcheting movements of their fangs, in which the left or right fangs alternately push the prey inwards.

Post-feeding Phase

Post-feeding phase involves muscle recovery. Once a prey has been successfully swallowed, the snake readjusted the jaw via a characteristic gape (or 'yawn'), whereby it opened the mouth wide. This behaviour was accompanied by multiple tongue flicks. Snakes exhibited gape display and tongue flicks frequently for 5–15 min. All four feeding snakes lifted the front part of the body above substrate, presumably to aid with peristalsis.

Temporal Analysis of Prey Handling Behaviour

Data for temporal analyses are in Table 2. Pre-capture phase showed the shortest of the prey handling time (range 17.09–495.01 sec; mean = 186.58 sec; ± SE 109.28). Feeding and post-feeding phase demonstrated a near equal mean prey handling time of 822.62 sec (range 433.08–1336.22 sec; ± SE 191.48) and 708.39 sec (range 392.16–1079.06 sec; ± SE 175.07), respectively. The ingestion process itself did not take as long as prey evaluation. Ingestion time period range was 259.19–1313.10 sec (mean = 580.39 sec; ± SE 247.43). The pre-capture phase took ca. 10.9% of handling time, while the feeding phase took 47.9% and post-feeding phase, ca. 41.2%.

Table 1. Nomenclature for predatory ethogram of *Tropidolaemus subannulatus* utilised in the current study. Modified from Danaisawadi *et al*. (2016).

Predatory phase	**Functional category**	**Behavioural display**	**Description**
Pre-capture	Detection	Head shift	Head tilts towards direction of prey
		Eye fixation	Stares at prey
	Approach	Approach	Head moves towards prey
Feeding	Capture	Strike	Strikes at prey and either lets go immediately after envenomation or prey continued to be held in the mouth
	Prey evaluation (Dead or alive)	Wait	Stays motionless
	Swallow	Upper jaw retraction	Transports prey body into oesophagus
Post-feeding	Muscular recovery	Gape	Opens mouth widely
		Tongue flick	Protrudes and withdraws tongue

Pre-capture	Prey introduction into enclosure
	↓
	Head Shift + Tongue Flick
	↓
	Approach
	↓
Feeding	Strike
	↓
	Wait
	↓
	Upper jaw retraction (Swallow)
	↓
Post feeding	Gape + Tongue Flick

Figure 2. Flow chart of predatory behaviour observed in *Tropidolaemus subannulatus*.

Figure 3. Female *Tropidolaemus subannulatus*, TROS 002 swallowing prey, using left fang to pull prey towards oesophagus.

Figure 4. Female *Tropidolaemus subannulatus*, TROS 002 swallowing prey using right fang to pull prey towards oesophagus.

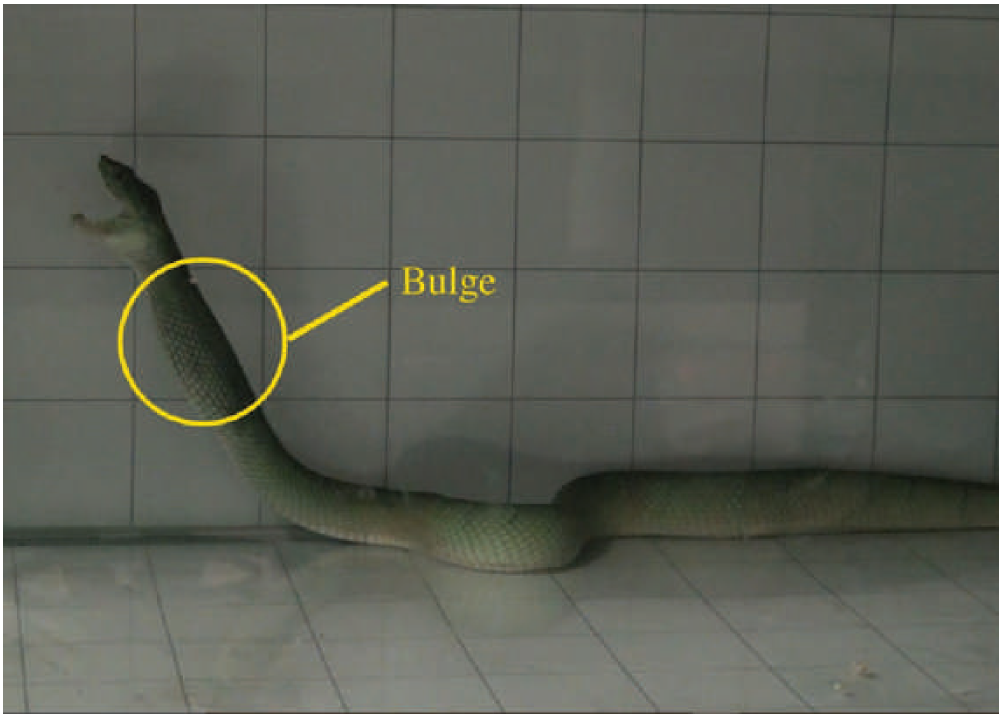

Figure 5. Female *Tropidolaemus subannulatus*, TROS 002 showing gape behaviour while lifting its anterior portion of the body part to aid in peristalsis to push down the food bolus (displayed in image as a bulge).

Table2. Mean and standard error of time length (seconds) of predatory phase in four female *Tropidolaemus subannulatus.*

Individual	**Morphometrics**		**Life Stage**	**Predatory phase time duration (s)**		
	SVL (cm)	**Mass (g)**		**Pre-capture**	**Feeding**	**Post-feeding**
TROS 001	44.70	45.0	Near Adult	47.12	852.07	427.3
TROS 002	57.6	95.0	Adult	17.09	433.08	1079.06
TROS 006	61.0	120.0	Adult	187.10	1336.22	935.03
TROS 009	51.60	84.0	Adult	495.01	669.12	392.16
			Mean	186.58	822.62	708.39
			SE	109.28	191.48	175.07

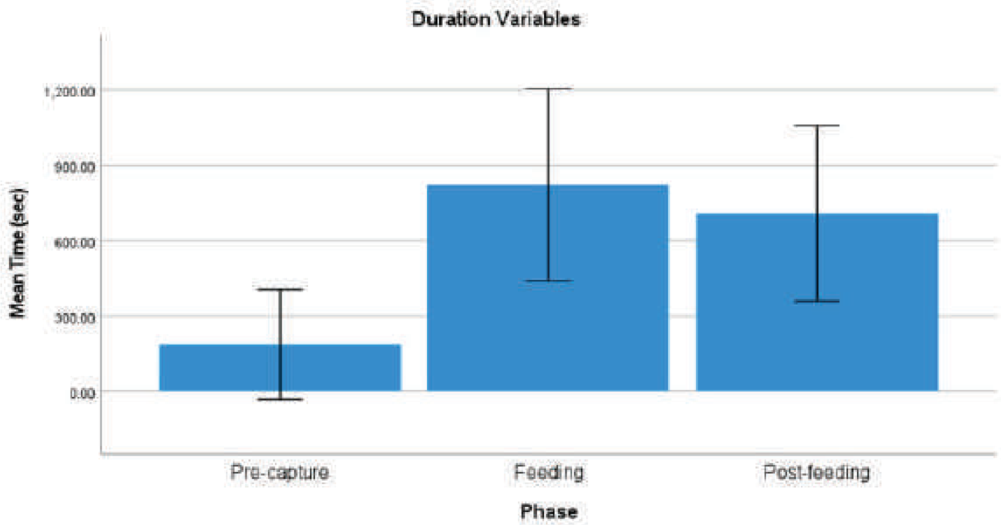

Figure 5. Mean prey handling variables in *Tropidolaemus subannulatus*, with standard deviation.

DISCUSSION

The behavioural analysis presented here reveals that individual snakes show slightly different feeding behaviours, albeit with several common components. A total of four out of 15 feeding behaviour experiments were completed, with the snakes successfully striking, killing and ingesting prey. A majority of individuals that did not feed displayed no interest in the prey, ignored the prey close to them, even with direct physical contact as the prey roamed in the tank. Snakes are known to exhibit variable feeding behaviours, depending on types of prey and circumstances of the encounter (Mori 1991, Mehta 2003, Danaisawadi *et al.* 2016). Visser (2015) reported that the feeding frequency in *Tropidolaemus subannulatus* can vary between 2–5 months, based on observations in captivity. Therefore, it is possible that individuals in the current experiment may have fed in the wild prior to being caught. Additionally, one test individual killed its prey but did not attempt to swallow it, perhaps perceiving the prey as a threat.

During the pre-capture phase, three of the four individuals displayed 5–6 tongue flicks before striking their prey. All snakes in these experiments did not change position but waited till the prey moved in the direction of the snake's head. In each case, the strike was initiated when the prey was approximately 10 cm away from the snakes' head. Cundall (2002) discovered that up to 47% of strikes by rattlesnakes (*Crotalus*) resulted in neither fang penetrating the prey, and that increase in the distance of the strike increases the probability of missed fang contact or penetration. The presumed consequence is that vipers prepare for predatory strike only when prey is in close range.

The feeding phase is one of the lengthiest and presumably important phases for the focal species. Upon striking and presumably envenoming the prey, they either let go of the prey immediately (one of four observations) or hold it in the jaws (three of four observations). One of the feeding females, TROS 001 envenomated and released its relatively small prey. Lillywhite (2014) stated that snakes usually retain a grasp on smaller prey and persist in holding it in the mouth firmly, till the prey stops struggling, before swallowing it. However, in the present case, the newborn and live young of *Rattus norvegicus* offered during the experiment was subequal to or smaller than bird or mammal presumably consumed in the wild (Stuebing *et al.* 2014). It is assumed that the snake ensures that prey is dead in order to safely feed, as it was observed that in 50% of the successful feeding observation (2 out 4), the prey displayed muscle twitching several minutes after being envenomated and following collapse. The time interval between prey mortality and ingestion also varied between individual snakes. Two individuals started ingesting prey under 5 minutes after envenoming, while the other two began ingestion about 120 minutes after envenoming the prey.

Post-feeding phase began once prey had been successfully swallowed completely, which involves muscle recovery (gape) and tongue flicking. It had been recorded that the anterior and posterior excursions of the palate and maxillary bones in vipers surpass those in other non-viper clades (Lillywhite 2014). There were little to no tongue flicks observed in all snakes before a strike, a somewhat furtive behaviour, as it waits to ambush their prey. The tongue flicking reportedly enables the transfer of molecular particles from the environment to vomeronasal organ, located in the roof of the mouth (Halpern and Kubie 1980, Kahmann 1932, Chiszar *et al.* 1982). After swallowing the prey, an open-mouthed gape was observed and accompanied by high rates of tongue flicks, lasting between 5–15 min. Chiszar and Radcliffe (1976) suggested that the tongue flicks may allow the snake to detect other possible prey which might occur in the vicinity.

The handling time of prey during the pre-capture phase appear to be the shortest among the three phases. Feeding and post feeding period took a relatively long time to complete. It is crucial for snakes to confirm that their prey is dead before consuming it. A failed killing can result in energy wasted, in which the effect can be significant for sedentary species, such as pit-vipers.

Prey types, such as newborn and young fuzzy *Rattus norvegicus* are relatively small, weighting about 10 g. Studies had shown that the strength of venom can be age-dependant (Chippaux *et al.* 1991) or show significant geographical variation as a result of diet (Daltry *et al.* 1996). It was found that coagulant activity in venoms is related to age and in some species, decrease in in coagulant activity increased with snake age (Bonilla *et al.* 1973, Kamiguti and Hanada 1985, Gutierrez *et al.* 1990, Chippaux *et al.* 1991). Ontogenetic relationships between snake size and prey release proportion remain untested due to sample size. Future studies are required on this species to understand the age-related behaviour concerning predation in the present species.

These observations made *ex-situ* with a sample of three adult females and one sub-adult female *T. subannulatus* provide new information on prey handling behaviour of a relatively common venomous snake in Borneo. The species displayed eight common predatory behaviours (head shift, eye fixation, approach, strike, wait, upper jaw retraction, gape, and tongue flick) in three different phases, namely pre-capture, feeding, and post-feeding phases during feeding experiments with new-born pinkies and fuzzies of *Rattus norvegicus*. In the pre-capture phase, which is the temporally the shortest, there are head shifts, eye fixation and head movement towards prey. During the long feeding phase, actions involve strike, wait to ensure prey death, and swallowing of prey. Post-feeding phase is a process of muscular recovery, followed by high-rate tongue flick that can last up to 15 min. The feeding behaviour of *T. subannulatus* is similar to that of the Indian trinket snake, *Coelognathus helena* hatchlings which kill house mice, *Mus musculus* via constriction and assessed it to confirm whether are dead prior to swallowing (Mehta 2003). This is fundamentally different from the Snail-eating snake *Pareas carinatus* which directly extract and swallow their prey (snails), regardless of the prey condition (Danaisawadi *et al.* 2016). Considering none of males and majority of the juveniles display an interest in prey offered during the feeding experiments, further research is required, with more prey choice and perhaps a longer pre-experiment period. The study by Danaisawadi *et al.* (2016) revealed variation of feeding temporal pattern based on prey types. Understanding the foraging and prey-handling behaviours are important and has the potential to provide deeper understanding on evolutionary fitness, as well as the biotic and abiotic factors which interacts with the focal species.

Acknowledgements: This research was funded by a Niche Research Grant from the Ministry of Higher Education, Government of Malaysia (NRGS/1087/2013(01), and administered by the Research Innovation and Enterprise Centre, Universiti Malaysia Sarawak. A debt of gratitude is due to our helpful colleagues and friends: Wong Jye Wen, Izneil Nashiq, Veronica Leah, Awang Khairul Ikhwan, Lorenzo anak Martin and Vanessa anak Martin provided us with endless assistance and support during the study despite multiple hurdles. We would like to acknowledge the Institute of Biodiversity and Environmental, UNIMAS for providing facilities, particularly Mohd-Azlan Jayasilan and Rahah binti Yakup. Deepest appreciation is due to the Sarawak Forest Department and Sarawak Forestry Corporation for issuing the research permits ((127)JHS/NC-CD/600-7/2/107; Park Permit NO. 69/2018 and SFC.PL&RS/2020-022; Park Permit NO.WL 32/2020), for which we are grateful to Zolkipli bin Mohamad Aton, Nurzehan Ramji, Melvin Gumal, Paschal Anak Dagang, Stephen Antang and Siti Wahdaniyah. Handling of animal protocol follows Animal Ethics Committee, Universiti Malaysia Sarawak, requirements (permission 2019–2020, dated 2 October 2019). We would like to thank Evan Quah for reviewing the manuscript, as well as Chong Kang Cherng for providing prey items for the experiments.

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