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Population Density and Morphometry Analysis for Sex Determination in *Varanus salvator* from Bangkachao, Samutprakran Province

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ABSTRACT

The study investigated the population density and morphometric analysis for sex determination of the water monitor (*Varanus salvator*) in Bagkachao, Prapadaeng, Samutprakran province, using mark capture-recapture during January 2012 to December 2012. Twenty six live-traps were placed every month (286 trap-days in total) and each reptile was tagged with a passive integrated transponder (PIT tag). Forty one lizards were recaptured (15.86% trap success). The population size was estimated using the Mark program software at 84 ± 50 individuals (min 47, max 306). The population density was 25.85 ± 15.38 lizards per square kilometer (0.258 ± 0.153 lizards ha^{-1}). Analysis of the captured specimens indicated an external sex difference in the eye-to-ear length (EEaL) ($P < 0.05$), with a mean (±SE) of 31.32 ± 1.19 in females and 35.36±1.29 in males. No juveniles were captured. The captured population structure ratios for sub-adult:adult consisted of 1:4.8, with a 1:1.6 sex ratio, and a 1:1.5 sub-adult sex ratio and 1: 1.12 adult sex ratio. The results indicated that *V. salvator* has not reached population saturation due to the reptile's habit of moving from one place to another to forage. This behavior allows several individuals to share the same space during the same period, depending on the integrity of the area and the habitats used by different-aged individuals. Moreover, mortality from various causes in the area revealed a declining population. Consequently, a proper policy is needed for the conservation of this reptile in the study area.

Keywords: Passive integrated transponder, population density, morphometry, *Varanus salvator*

INTRODUCTION

There are 71 species of varanid lizards globally, including 4 species in Thailand-Dumeril's monitor (*Varanus dumerilli*), the roughnecked monitor lizard (*Varanus rudicollis*), the Bengal monitor (*Varanus begalensis*) and the water monitor (*Varanus salvator*) (Lauhachinda, 2009, Koch *et al.*, 2013 Uetz and Hošek, 2015). In their natural habitat, water monitors play an ecological role as a scavenger. They are predators who control the populations of other animals. Water monitors can be found in almost every part of the country, from the upper central, lower northeast, to the south of Thailand (Cota *et al*., 2009). They live near fresh water, salt water or brackish water such as in the mangrove forest zone, agricultural zones, marshes, in canals, human community zones and human living zones (Lauprasert, 1999). In Thailand, during the past few years, the living zones of the reptile have extended into human communities due to the increase in numbers of water monitors. Numbers of this reptile increased sharply in Samut Prakarn, Samut Songkhram, Samut Sakhon and Ratchaburi provinces, where the water monitors have harassed the local people by digging up food scraps in garbage cans and by eating domestic animals such as ducks, chicken and fish (Matichon, 2011; Cota *et al*., 2009). These problems have resulted in individual specimens being chased and killed Suekamnurt (2007). The reason for killing partly came from the negative attitude and belief in this animal–"เหี้ย" (h⊠ə), the Thai name of water monitors, means "extremely bad". It is, moreover, a very impolite word used to insult other people (Cota *et al*., 2009). However, this problematic situation has not been solved appropriately due to the lack of survey information and population estimates of water monitors in Thailand.

The current project was undertaken in a large area classified as a green zone in urban Bangkok where there has been a conflict between people and water monitors. The study aimed to quantify the population size and density, sexuality differences and the annual population structure of water monitors. This information can be applied to the preservation of species in the varanid family or it can be used to draft a potential management plan for this reptile in the future.

MATERIALS AND METHODS

Study area

The data collection took place in Bang Krachao sub-district, Phra Pradaeng district, Samut Prakarn province, which covers an area of about 200 ha. It is a part of the Bang Krachao area in a green conservation zone (shaped like a lasso or a pig's stomach) bounded by 15 km of the Chaophraya River, which has legislated protection (Royal Forest Department, 2011; Kasetsart University, 2013; Homchan *et al*., 2011). It extends from latitude 13° 39′ 10″ N to 13° 42′ 30″ N, and from longitude 100° 32′ 20″ E to 100° 35′ 27″ E, and has an annual average temperature of 30.4 °C and an annual average rainfall of 106.6 mm. Most of the area has been abandoned or is used for agricultural purposes such as fisheries, orchards with ditches, home-grown vegetables, flower gardens and garden trees, wood lots for economic purposes and for environmental conservation (Khooranarak *et al*., 2013). Moreover, it is a biologically significant area due to its species variety associated with its three ecosystems spanning fresh, salt and brackish water. (Green World Foundation, 2015).

Field data collection

The study area was divided using a geographical grid system consisting of six grids (1,000 × 1,000 m) in total. Each grid was sub-divided into four sub-grids (500 × 500 m); furthermore, as three grids were, inaccessible (No. 1, 4 and 8 due to access problems), the a final study area had 13 sub-grids. Two cages (each 200 cm long × 50 cm wide × 50 cm high) (Figure 1) were placed in each sub-grid approximately 200-300 m apart (Figure 2). There were 26 trap-days during each month, resulting in 286 trap-days in total from January to December 2012 (no trapping in September). Each cage was checked twice a day (9:00 am and 3:00 pm). Once trapped, each monitor lizard was tagged with a passive integrated transponder (PIT tag; 2 mm × 12 mm) which was injected using a needle (No.12) into the center of the back between the shoulders (according to Ferner, 2007). Each tag had a unique microchip identification code number that enabled the capture history for each tagged animal to be recorded and for recaptured animals to be identified. Morphological measurement was undertaken using a pair of Vernier calipers and each recaptured reptile was weighed according to Lauprasert (1999) and the sex and age class were identified following Shine *et al*. (1998). Following measurement, each reptile was released where it had been trapped.

Figure 1 Cage (200 cm long × 50 cm wide × 50 cm high).

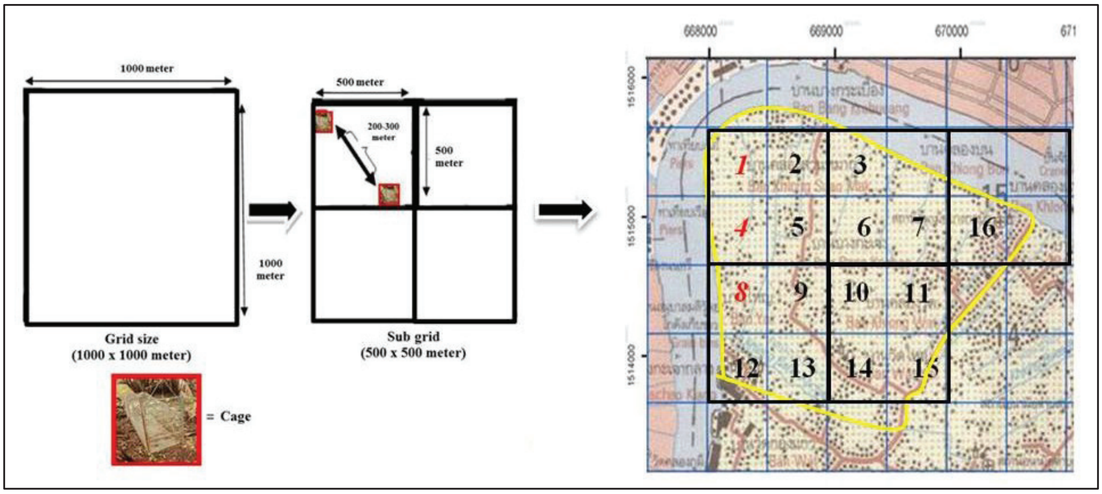

Figure 2 Geographical grid system (1,000 × 1,000 m) used to divide the study area with the grid sub-divided into four sub-grids (500 × 500 m), covering 13 sub-grids (excluded No. 1, 4 and 8 due to access problems).

Data analysis

Population density

The population size was determined from the capture data using the MARK software program (Version 7.1; Gary White; Colorado State University, Fort Collins, CO, USA) with the closed population model and the Lincoln-Petersen Estimator (White, 2008):

$$\hat{N} = \frac{n_1 n_2}{m_2}$$

$\hat{N}$ = Estimate number

n_1 = Number of captured lizards the first time

n_2 = Number of captured lizards the second time

m_2 = Number of marked lizards the first time and the second time

The data capture history of each lizard in the study period was examined to detect probabilities from the following eight models: Mo (probability of capture and recapture of equal value), Mh (probability of capture and recapture dependent upon the heterogeneity among individual animals), Mb (probability of capture and recapture dependent upon the behavior of animals), Mt (probability of capture and recapture variation with time), Mbt (probability of capture and recapture dependent upon the behavior of animals and the variation of time), Mbh (probability of capture and recapture dependent upon the behavior and the heterogeneity among individual animals), Mth (probability of capture and recapture dependent upon the variation of time and the heterogeneity among individual animals), and Mbth (probability of capture and recapture dependent upon the behavior and the variation of time and the heterogeneity among individual animals).

The best model to estimate the population was determined using the lowest

AIC value of Log Likelihood not exceeding 2. (Cooch and White, 2008; Chim and Diong, 2013; Cooch and White, 2013, 2014).

The population density in the area was calculated based on the population size according to the formula D = N / A (Karanth and Nichols, 1998).

Different morphology of sexuality

Measurement data were used to determine differences between the sexes at different ages. The average values were compared using a *t*-test and discriminant analysis in the SPSS software Version 16 and is shown below as a linear equation (Vanichbuncha, 2011):

$$D = a_i + b1x1 + b2x2 + \ldots\ldots. + bipxip$$

D = Discriminant score
bi = Discriminant coefficients,
xi = Discriminant variables
p = Number of variables
a_i = Constant

Population Structure Analysis

The sexual identification was classified and the age range was graphed to describe the relative proportion of the population structure as juveniles, sub-adults and adults using SPSS Version 16.

RESULTS AND DISCUSSION

Population size and density

In total, 43 water monitors were captured, consisting of 41 individuals with 2 being caught twice. The capture success rate was 15.86%. The rate of average monthly capture was 3.73±1.35 with the highest capture number being 7 in February while the minimum was 2 in May (Figure 2). Using the Mark program, the annual estimate population size from Mb (assuming that the lizards were on the move indefinitely (Traeholt,1993) was 84±50 with a minimum estimate of 47 and a maximum of 306 (Table 1). The population density was 25.85±15.38 monitor lizards per km^2 (0.258±0.153 per ha). The minimum estimate was 14.46 monitor lizards per square kilometer (0.145 per ha) and the maximum equated to 94.15 monitor lizards per square kilometer (0.942 per ha) (Table 2).

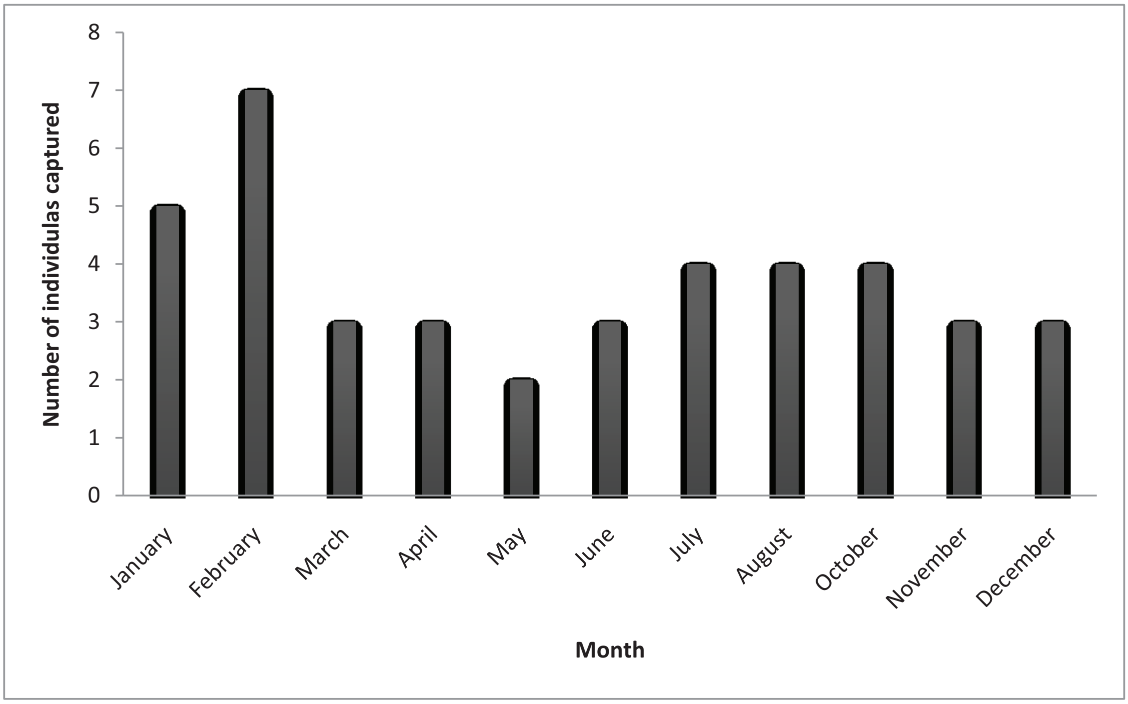

Figure 3 Number of water monitor lizards captured between June and December 2012 (excluding September).

Table 1 Annual estimated lizard population from the MARK program using eight models.

Model	$\hat{N}$	Standard Error	95% Confidence Interval	AIC
Mo	397	270	136-1377	0.573
Mb	84	50	47-306	1.554*
Mh	398	271	136-1378	1.770
Mbh	100	80	49-480	2.843
Mtb	41	0	41-41.000	14.838
Mt	334	225	117-1159	15.434
Mth	393	267	135-1360	16.102
Mtbh	44	9	41-97	16.840

Note: *Chosen for additional study.

Table 2 Captured and estimated lizard population density in the study area.

	Population size	Study area (km^2)	Population density in the area (lizard)	
			Per km^2	Per ha
Capture	41	3.25	12.62	0.126
Estimate	84 ± 50		25.85 ± 15.38	0.258 ± 0.153
Max. estimate	306		94.15	0.942
Min. estimate	47		14.46	0.145

The results of the study indicated the population size and density were low; there were 43 water monitors captured throughout the study, including 2 recaptures with a registered capture history. There was a tendency for non-registered lizards to be increasingly recaptured. This indicated that the study area could support a larger water monitor population and suggested gradual growth which would be indicated when the number of recaptured lizards is consistently substantial (Akcakaya *et al*., 1999): An ability to support the population of the area (K, Carrying capacity) can be demonstrated by the variety of resources and efficiency to support the population. However, if it is found that the population has decreased, this indicates that the population exceeds the carrying capacity or there is over-population. Such factors are the result of the birth rate, natural mortality rate, migration, numbers killed by other lizards and the environmental factors that affect the survival of animals. According to Molles (2013), patterns of population growth takes many forms which may possibly start with a slowing down of exponential growth leading to the appropriate environment so that the population then increases more rapidly. During the study period, the capture rate in summer was low while in the rainy season and winter, it was quite high, perhaps because the rains continue through winter and there is more food from the effects of rainfall and the animals are fully fed (Traeholt, 1998; Uyeda, 2009; Uyeda *et al*., 2012). The report showed that the source of waste and the amount of water during the rainy season will affect the selection of the area because the normal activity of water monitors involves eating carcasses and hunting pests (Gaulke *et al*., 1999). Populations of the Varanid family living in tropical areas are influenced by the temperature and the abundance of food during the year (Wikramanayake and Dryden, 1993).

The findings were in accordance with the results of Suekamnurt (2007) who studied the ecology of water monitors and the effects of land use in the Laem Pakbia Environment Research and Development Project which found that the population density estimate was 38.94 per km^2 (0.389 per ha). In contrast, the results of the current study showed a marked difference from the results reported by Rashid and Hoong (2004) who studied the population ecology and management of water monitors, *V.salvator* (Laurenti) in the Sungei Buloh Wetland Reserve, Singapore.

They found 181 water monitors in the area and their population estimate was 3.9±0.25 per ha. The differences in numbers might have been caused by the size of the study area or might depend on limiting factors in each area such as the amount of food, competition. However, National Parks Board Singapore (2010) reported that there are strong laws and area protection management and severe punishment in order to maintain the richness of the Sungei Buloh Wetland Reserve. Differences may also be related to the behavior of the family Varanidae which is known to explore, capture and then consume their prey (Blamires and Douglas, 2004) which accords with Traeholt (1993) who concluded that water monitors usually change their habitat to find food and do not remain in any specific area. Rashid and Hoong (2004) reported that the movement of water monitor lizards is related to the migration of birds that flock together to nest. Gaulke *et al.* (1999) Auliya (2003) Gaulke and Huorn (2004) and Uyeda (2009) also discovered that water monitor lizards can live in a wide ranges of areas, from 50 to 9,000 square meters and that their home range is very dependent on the habitat and food source. However, most of their behavior is governed by the selection of habitat and micro habitat, with water sources and semi-aquatic areas being important along with very low temperatures as these affect activity, with warmer temperatures associated with aquatic environments and coastal areas (Wikramanayake and Dryden, 1993).

Morphology

The results regarding water monitor morphology were based on the sub-adults trapped (7 water monitors: 2 females, 3 males and 2 non-classified). The comparison test between females and males revealed no significant difference ($P > 0.05$). Measurement data collected could not pass Box's M test. Therefore, a discriminant score equation is not possible. Water monitor morphology in the trapped adults (33 water monitors: 17 females and 16 males), using only one characteristic eye to ear opening length (EEal) was significantly different ($P < 0.05$) between females and males (Figure 4) with a mean value of 31.32±1.19 mm in males and 35.36±1.29 mm in females (Table 3). The sex classification equation was D = TTL (-0.104) + EEaL (0.179) + TW (0.484) + EL (0.119) + EW (-0.038) + 6.329. The equation indicated among the 16 previous classified male water monitors, 14 would be classified as males and 2 as females while in 17 previously classified females, 14 would be classified as females and 3 as males. Thus, the average accuracy for both sexes was 84.8% (87.5% for males and 82.4% for females), which was identical to the original classification.

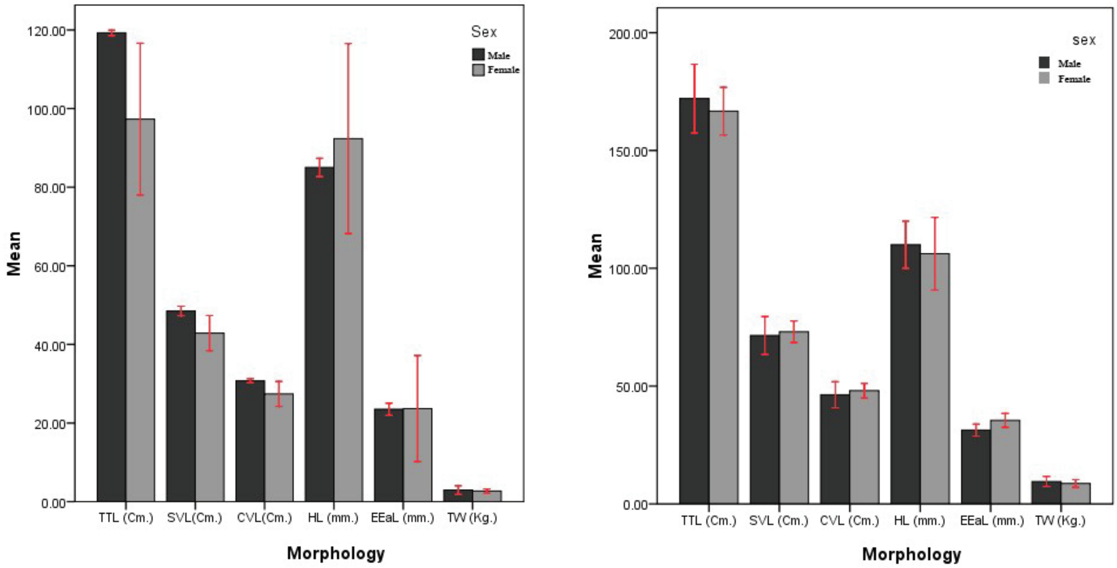

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Figure 4 Comparative morphology of sub adults (A) and adults (B) between males and females for Total Length (TTL), Snout-Vent Length (SVL), Head Length (HL), Eye-Ear Length (EEL), Coral-Vent Length (CVL) and Total Weight (TW).

Table 3 Significance testing of some morphological differences between male and female adults.

Morphology	Mean		*t*-Test	*P*-value
	Male (n=16)	Female (n=17)		
TTL (cm.)	171.61±7.015	166.68±4.74	.792	.434
SVL (cm.)	71.45±3.78	73.04±2.13	-.529	.601
CVL (cm.)	46.30±26.10	48.038±14.19	-.732	.469
HL (mm)	109.99±4.69	107.68±6.97	.271	.788
EEaL (mm)	31.32±1.19	35.36±1.29	-2.28	.030*
TW (kg)	9.50±1.00	8.59±0.711	.749	.460

Note: *Significant values (*P<0.05*)

These results showed that there was no significant difference between sub-adult females and males and so it was impossible to use the equivalent matrix hypothesis in Box's M test. The current results involving only a single difference between male and female adult water monitors differed from Suekamnurt (2007) who reported 3 differences among 20 male and female water monitors, namely, Base of Tail Circumference Length (LCL), Nostril Length (NL) and Eye Width (EW). It is possible that the results from the two studies differed due to the fact that a smaller number of water monitors was captured in the current study and the analysis techniques were not the same. This is supported by Thompson and Withers (1997) and Smith *et al*. (2007) who reported that the number of individuals in the sample affects the classification of independent or small groups, and this could result in major influences on the morphological classification by sex group, ecological group and physical size. Thompson and Withers (1997) studied sex classification in 18 families of monitor lizards in Australia using a Stepwise Discriminate technique, evaluating F-values to distinguish physical appearance. They reported different morphological characteristics for the classification of each type of monitor lizard. For example, in *V. caudolineatus*, the Thorax–Abdomen Length (TA) and Head Length (HL) Smith *et al*. (2007) who reported *V.mertensi* and *V.mitchelli*, the significantly different morphology was found in the Head Length (HL) and Forlimb Length (ForL).

Overall, the current study showed that both female and male water monitors are morphologically familiar but that only some morphological characters can be used for classification, suggesting that water monitors exhibit sexual dimorphism. This consequence is consistent with the research on lizard groups which stated that this type of animal mostly is distinguished by head size and body size, with head size generally bigger in males than females and the hind limb size normally larger in females. These appearance differences are clearly visible in the Varanid family such as *V.indicus V.begaensis V.komodoensis* (Olsson and Madsen, 1998; Olsson *et al*., 2002; Lawwell, 2006; Frýdlová, 2013).

Population structure

The age structure of the 41 lizards was classified as 0 juveniles, 7 sub-adults and 34 adults, with a ratio is 0: 1: 4.8, respectively. The sex structure of the 41 lizards was classified as 18 females, 21 males and 2 non-identified lizards; with a sex ratio of 1:1.16 for male to female, respectively, excluding the non-identified individuals. Sub-adults were classified as 2 females, 3 males and 2 non-identified lizards with a sex ratio 1:1.5, respectively, excluding the non-identified individuals. Adults were classified as 16 females and 17 males (sex ratio of 1: 1.12, respectively).

The current results were similar to those reported by Suekamnurt (2007) where the age ratio was 1:3, the sex ratio was 1:1, the sub-adult sex ratio was 1.5:1 and the adult sex ratio was1:0.87. Rashid and Hoong (2004) reported a sex ratio of about 1:1, with 31 % of females being sub-adults and 61% of males being adults, when typically, the ratio of males to females based on the theory of reptiles should be 1:1(Lauhachinda, 2009).

Miller and Spoolman (2012) in their study on monitor lizards considered that the number of males and females in the juvenile, middle-aged and old groups will determine the population growth and decline. The results of the current study trapped no juveniles and the number of sub-adults recorded was very small compared to the adults trapped. Therefore, the lizard populations in the study area seemed to be declining, with fewer juveniles and sub-adults to replace the adults over time. The declining population in the results may have been due to a technical flaw as the cage mesh size was larger than the body size of juveniles, so that juveniles were not contained in the traps. It may also have been due to the natural behavior of juveniles and sub-adults who avoid the space used by adults and also avoid competing with adults for food, as well as being vulnerable to being hunted and subjected to natural disasters in the area. Following Pianka (1997), it was concluded that the reptiles have generally high fecundity, but this is accompanied by a high death rate and low survival rate after the juvenile period. A survey of biodiversity in the study area found that there were several predators, for example cobra (*Naja kaouthia*), Golden tree snake (*Chrysopelea ornata*), Reticulated python (*Malayopython reticulatus*), Collared Falconet (*Microhierax caerulescens*), Stork-billed Kingfisher (*Pelargopsis capensis*) according to Green World Foundation (2015). Similarly, Karunarathna *et al*. (2008) reported predators who preyed specifically on juvenile water monitors, including the Brahminy Kite (*Haliastur indus*) and Shikra (*Accipiter badius*). Sub-adults could successfully avoid being prey because they were well camouflaged. Furthermore, Ghimire and Shah (2013) reported that *V.flavescen* individuals were killed by dogs (*Canis sp*.) in their study (Figure 5) and were also identified as road kill. This agreed with Duengkae and Chuaynkern (2009) and Karunarathna *et al*. (2012) who reported juveniles of both *V. salvator* and *V. begalensis* as road kill. Moreoever, natural disasters that cannot be predicted may reduce the survival rate of eggs and reduce future numbers of sub-adults. In the current study period, from May to December 2011, many areas of the country, including the study area, were affected by tropical storms which caused flash flooding and the study area was subsequently subjected to the overflow inundation (Thailand National Statistical Office, 2011).

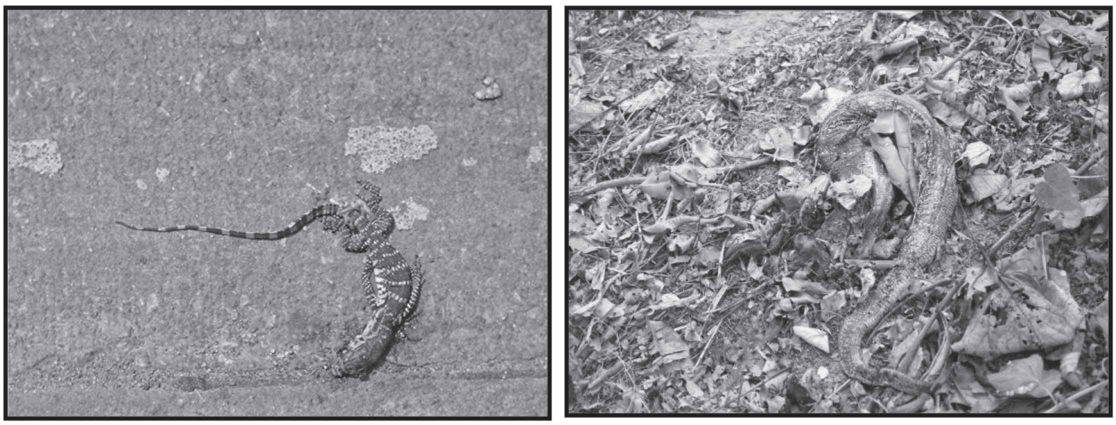

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Figure 5 Mortality factors in the study area (A) a juvenile was kill on road (B) a sub adult was bitten by dog.

CONCLUSION

The study involved 286 trap-days, resulting in the capture of 43 water monitors of which 41 were marked lizards and 2 were recaptures. The population density was estimated at 25.85±15.38 lizards per km^2 (0.258±0.153 lizards per ha) ranging from 14.46 to 94.15 lizards per km^2. Initially, the number of water monitors caught was low but the capture of unmarked water monitors gradually increased while the re-capture rate stayed low. This suggested that area was underpopulated and that the area could support more lizards. This inference is a consequence of water monitor behavior as individuals move to new areas to find food. There were morphological differences between males and females that were distinguishable in adults. Both juveniles and sub-adults were captured in small numbers compared to adults. This suggested a declining population due to several factors such as the limitation associated with the natural behavior of the reptiles and high mortality in the area from many causes.

This study can be applied to water monitor management in other places. However, there should be improvements made to the number of traps and their size. Permanent traps should be placed in less accessible locations to cover the whole study area, as well including data collection on the abundance and types of food, nesting site selection and threat factors. Monitoring individuals in every age range by radio transmitter tagging would allow movement and area usage to be tracked during different seasons. The results of these improvements would be useful in studying water monitors and the long-term impacts of human beings on the lizard population, which could then be included in the development of conservation or population control measures in the future.

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