RESEARCH ARTICLE
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Sexual Size Dimorphism and Reproductive Cycle of the Little File Snake *Acrochordus granulatus* in Phangnga Bay, Thailand

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ABSTRACT: A total of 119 little file snakes (*Acrochordus granulatus*), collected at Phangnga Bay from February 2002 to August 2003, were studied. The snakes were divided into four groups according to their sex and reproductive stage: 23 juvenile males, 19 adult males, 32 juvenile females and 45 adult females. T-test and discriminant function analysis were used to analyze the data on sexual size dimorphism. Significant differences (t-test, $p \leq 0.05$) in 11 of 14 morphological characters were found between the sexes of adult snakes. Weight, head length, head width, distance between eyes, snout to gape length, mouth width, snout to vent length, neck girth, body girth and tail girth were larger in adult females than in adult males while vent width was greater in males. In juvenile snakes, only two morphological characters, mouth width and vent length, were significantly different. Results from discriminant function analysis yielded an equation for predicting the sex of adult little file snakes with the original grouped case correctly classified at 98.3%.

Reproductive data indicated that the breeding season of *A. granulatus* begins in July. From July to December, the testicular volume increased, surpassing that observed from January to June. Following an increase in size of follicles to vitellogenesis, ovulation was observed in September. The embryos were first observed in January and young snakes of about 360 - 400 mm snout to vent length were first caught in June.

KEYWORDS: *Acrochordus granulatus*, marine snake, sexual size dimorphism, reproductive cycle.

INTRODUCTION

The family Acrochordidae contains only one genus with three species, the Arafura file snake (*Acrochordus arafurae*), the Elephant's trunk snake (*A. javanicus*) and the little file snake (*A. granulatus*). These snakes are distributed in South Asia, Southeast Asia and Australia. *A. arafurae* and *A. javanicus* are freshwater snakes, whereas *A. granulatus* occurs in a variety of aquatic habitats ranging from brackish coastal habitats to the sea[1]. Of these, two species, *A. javanicus* and *A. granulatus*, have been reported in Thailand[2-4]. *A. granulatus* is nocturnal and frequently found near river mouths at depths of 4 to 20 meters. This species feeds on gobies and occasionally on marine crustaceans[5-8].

A. granulatus has small scales with tubercle-like keels, and a stout and compressed body[9-10]. Although, in many species of animals, males and females differ in body size, shape, and color (either because of sexual selection or ecological divergence between the sexes) most snakes do not show extreme sexual dimorphism[11,12]. The sexes are not easily distinguished on the basis of external features and the reproductive biology of *A. granulatus* is not well understood[1,5,13]. Therefore, the emphasis of this study is on the sexual dimorphism and reproductive biology of *A. granulatus* found at Phangnga Bay, Thailand.

MATERIALS AND METHODS

Study Site

Phangnga Bay is located in Southern Thailand (Fig 1). It covers parts of three provinces; Phangnga, Phuket and Krabi. The 1,960 km^2 bay with water depth between 1 to 20 meters has been classified as one of the most biologically productive and ecologically important bays in the world. Mangrove forests, seagrass beds and reefs are major habitats along the coast of the bay. Throughout the year, in the bay and along the shoreline, marine snakes are occasionally captured by local fishermen.

This circumstance provided a good opportunity to study sexual dimorphism and the reproductive biology of *A. granulatus* in the bay.

Methods

Specimens of *A. granulatus* were collected at 3 locations: [1] Ban Klong Khiean (N8.14042 E98.42471), [2] Ban Sam Chong (N8.29094 E98.50727) and [3] Ban Leam Sak (N8.28185 E98.60725) during February, 2002 to August, 2003. Floating seines, cast nets and fishing traps were deployed by the fishermen along the shoreline of these locations at the water depths between 2 and 6 m. These traps were monitored for *A. granulatus* during low tide periods.

The weight and 13 morphological characters were recorded for each specimen. Snout- vent length (SVL; the length from the tip of snout along the body to the vent opening), tail length (TL; the length from the vent opening to the tip of the tail), neck girth (NG; the length around the neck), body girth (BG; the length around the middle of the body) and tail girth (TG; the length around the base of the tail at the vent opening) were obtained using a tape measure (±1 mm). Head length (HL; the distance from the tip of the snout to the end of the lower jaw which is close to the neck), head width (HW; the highest distance between the lower jaws), distance between eyes (Eyes; the distance between the right eye and the left eye), snout to corner of the mouth length (SCL; the tip of snout to the corner of the mouth), snout to right eye length (SEL; the tip of the snout to the right eye), vent width (VW; the maximum width of vent opening) and vent length (VL; the maximum length of vent opening) were obtained using a vernier caliper (±0.5 mm). Maximum mouth width (MW; the maximum size of the mouth opening) was measured by inserting a plastic rod of known diameter (±1 mm).

The reproductive information was obtained from dead snakes found in the traps. The size of the testes and follicles of the ovary were measured by a micro-scale (±0.1 mm) under the microscope. Then, their

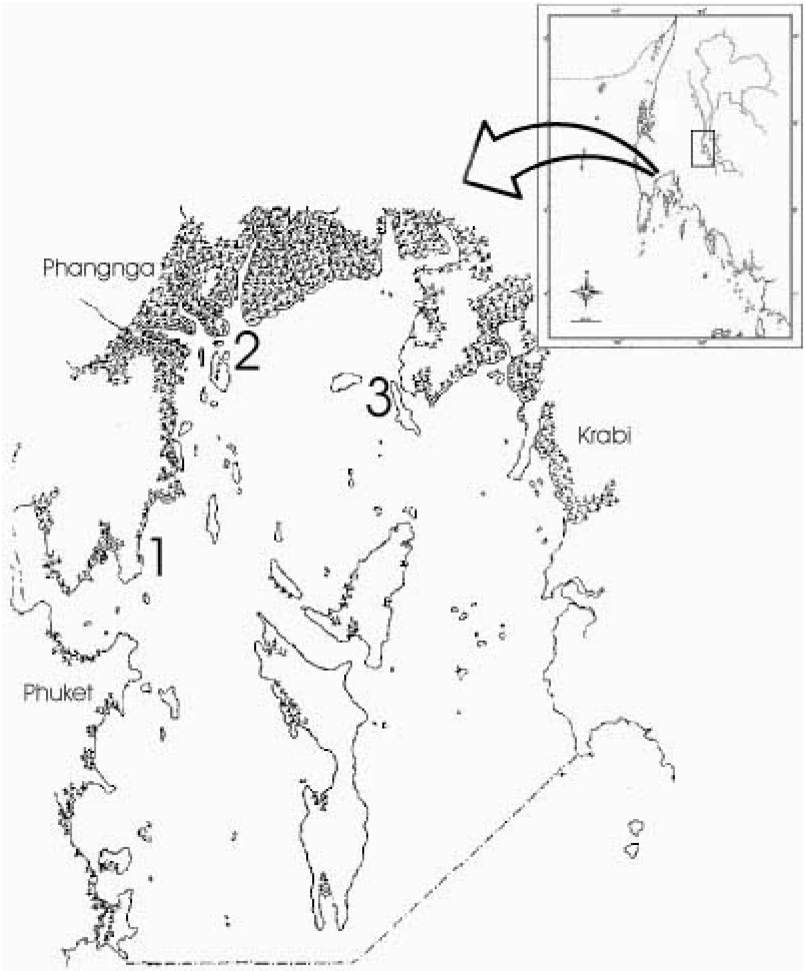

Fig 1. Phangnga Bay (08° 04´ - 08° 24´ N and 98° 04´ – 98° 39´ E): showing three locations at the northern part of the bay that *A. granulatus* specimens were collected.

volumes were calculated by mathematical formula and the stages of ovulation were recorded.

Morphological differences between the sexes of juvenile and adult snakes were compared by applying the *t*-test. Discriminant function analysis (DFA) was used to determine which variables discriminate between adult males and females, and to produce a centroid for each sex. However, the weight was not used for calculation due to the great variability resulting from feeding events and the reproductive condition of individuals[13 14]. The statistical analysis was performed on a computer using SPSS 10.00 for windows. To avoid size bias, all measurement data were transformed by logarithm[3] and divided by head length (HL).

Results

Sexual Size Dimorphism

The frequency distribution of 42 male and 77 female little file snakes is presented in Fig 2. The histogram shows that many females attain a larger size than males. Most male snakes captured had SVL between 551-600 mm while females were between 601-650 mm. The largest male found had a SVL of 690 mm and a total length of 765 mm, whereas the largest female had a SVL of 850 mm with the total length of 946 mm.

Breeding Season and Size at Sexual Maturity

To demonstrate the breeding season and size at sexual maturity of *A. granulatus*, the relationship of SVL to testicular volumes and follicle volumes are presented in Fig 3. Most males (n = 11) and females (n = 28) with SVL over 600 mm captured during July to December were reproductively mature as evidenced by enlarged testes and vitellogenous follicles. Only a few snakes captured during January to June with SVL over 600 mm had enlarged testes or follicles. This phenomenon indicates that the breeding season of *A. granulatus* at Phangnga Bay may start in July, or at the end of the monsoon season.

The graphs suggest that the snakes can be divided into 4 groups based on their sex and size at maturity. Snakes below 580 SVL are juveniles with sexually immature testes and ovaries whereas those above 580 are sexually mature adults.

The results of the *t* test on 14 morphological characters between juvenile males and females shown in Table 1A indicate significant differences only in mouth width (females larger than males) and vent length (males larger than females). Among adult males and females, 11 out of 14 characters were significantly different; weight, head length, head width, distance between eyes, snout to gape length, mouth width, snout to vent length, neck girth, body girth, tail girth and vent width (Table 1B). In all of the latter characters except vent width the adult females were larger than the adult males (Fig 4).

To calculate the size difference between males and females, the sexual dimorphism index proposed by Gibbons and Lovinch (1990)[15] was used (sexual size dimorphism (SSD) = [mean adult SVL larger sex / mean adult SVL smaller sex] – 1). This index was expressed as positive if females were the larger sex and negative if males were larger. The SSD index of *A. granulatus* for this study was 0.058.

Discriminant Function Analysis (DFA) of adult *A. granulatus* indicated highly significant discrimination (Wilks' Lambda 0.297; $p < 0.05$; canonical correlation 0.839). The eleven variables utilized in the discriminant function and discriminant scores showed sexes group functions as the following.

$$Y = 88.461(HL) - 50.317(HW) + 55.949(Eyes) + 55.116(SEL) - 35.658(SGL) - 3.198(SVL) + 153.555(TL) + 57.319(NG) - 57.063(BG) + 70.885(TG) - 249.749(MW) - 16.021$$

Where the Y scores were less than 1.42 snakes were classified as male, and where scores were more than 1.42 snakes were classified as female. The original grouped case correctly classified at 98.3%. Twenty specimens of unknown sex were tested correctly by this function, the sexes showed a significant difference between centroids (males 2380 and females -0.963; where units indicate discriminant scores).

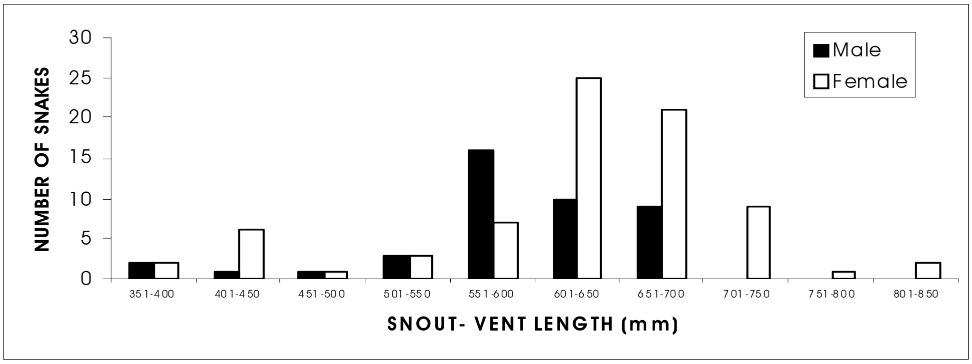

Fig 2. Frequency distribution of male and female snout-to-vent lengths of *Acrochordus granulatus*.

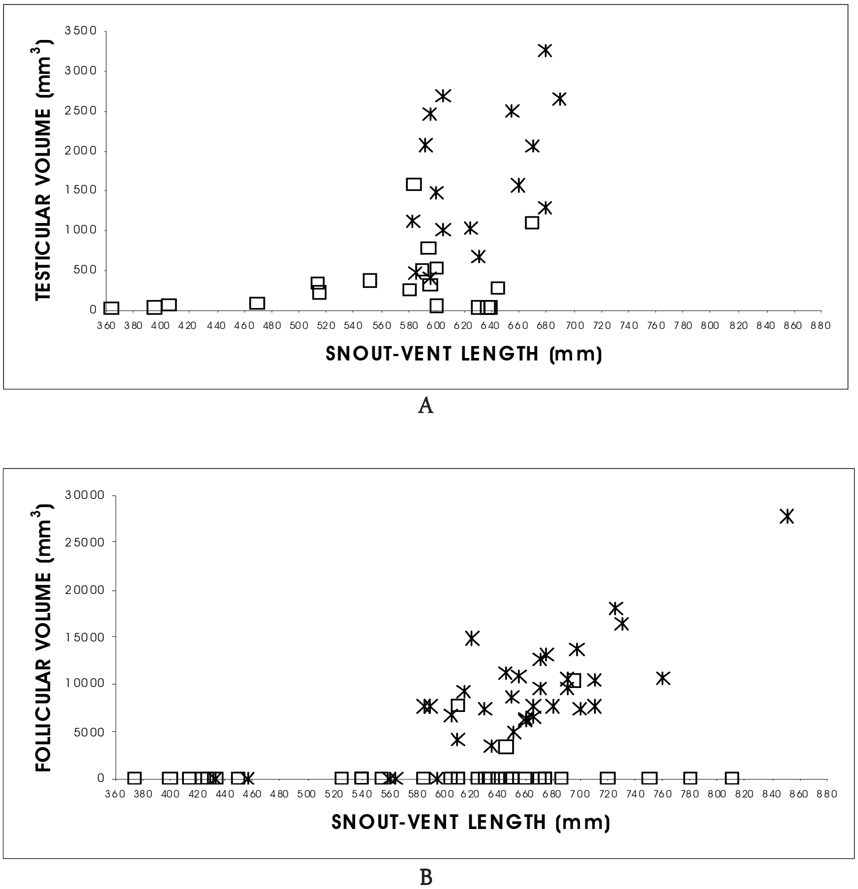

Fig 3. Plots of SVL versus testicular volumes (A) and follicle volumes (B) in *A. granulatus*. Squares (□) show size variations from January to June and asterisks (*) show size variation from July to December.

Table 1. Sexual size dimorphism in juvenile (A) and adult (B) *Acrochordus granulatus* from Phangnga Bay, Thailand. The table shows the mean values (± SE) for 14 morphological characters and the P-values from the independent-sample *t*-test.

Morphological characters	(A) Juveniles			(B) Adults		
	Male	Female	P value	Male	Female	P value
Sample size	23	32		19	45	
Weight (g)	73.55 ± 6.56	83.60 ± 7.68	0.324	104.72* ± 5.02	168.52* ± 8.28	0.000
Head length (mm)	15.62 ± 0.36	16.46 ± 0.44	0.171	17.78* ± 0.24	19.57* ± 0.27	0.000
Head width (mm)	10.09 ± 0.30	10.84 ± 0.26	0.067	11.11* ± 0.22	13.24* ± 0.30	0.000
Distance between eyes (mm)	6.17 ± 0.15	6.30 ± 0.18	0.601	6.95* ± 0.15	7.41* ± 0.09	0.014
Snout to corner of the mouth length (mm)	9.35 ± 0.25	9.50 ± 0.21	0.640	9.95* ± 0.21	11.18* ± 0.25	0.000
Snout to right eye length (mm)	4.43 ± 0.14	4.29 ± 0.09	0.441	4.86 ± 0.13	5.15 ± 0.10	0.092
Mouth width (mm)	16.65* ± 0.51	18.37* ± 0.51	0.020	18.73* ± 0.34	21.46* ± 0.25	0.000
Snout to vent length (mm)	548.39 ± 15.02	543.22 ± 14.59	0.880	648.63* ± 6.33	686.26* ± 7.09	0.000
Tail length (mm)	63.91 ± 2.06	62.66 ± 1.74	0.643	74.95 ± 1.34	73.67 ± 1.00	0.462
Neck girth (mm)	30.10 ± 1.03	30.96 ± 0.70	0.494	32.92* ± 0.84	36.71* ± 0.64	0.001
Body girth (mm)	52.44 ± 2.05	52.09 ± 2.11	0.908	56.89* ± 1.07	65.83* ± 1.56	0.001
Tail girth (mm)	21.74 ± 0.78	21.28 ± 0.65	0.651	24.82* ± 0.51	27.03* ± 0.52	0.004
Vent width (mm)	1.81 ± 0.13	1.57 ± 0.07	0.128	2.21* ± 0.11	1.87* ± 0.07	0.016
Vent length (mm)	2.21* ± 0.14	1.78* ± 0.11	0.026	2.19 ± 0.13	1.98 ± 0.10	0.230

* Significant difference at $p < 0.05$

Fig 4. Photograph showing head size difference between an adult female (upper, CUBMZR2005.1, SVL = 610 mm) and an adult male (lower, CUBMZR2005.2, SVL = 630 mm) of *Acrochordus granulatus*.

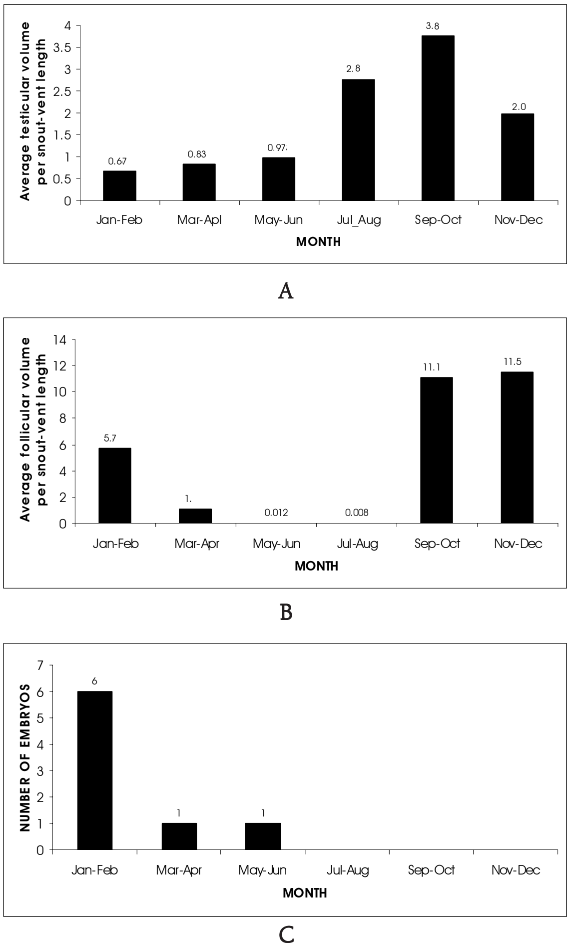

Fig 5. Monthly variation in the testes volume/SVL of males (A), follicle volume/SVL of females (B) and number of embryos occurring (C) in *Acrochordus granulatus*.

Reproductive Cycle

Reproductive data indicate that the breeding season of *A. granulatus* in Phangnga Bay begins in July. The average values of the testicular volume increased in July through October and remained elevated in November and December. For example, the testes volumes in September to October (3.8 ± 1.1 mm^3) were larger than in both July to August (2.8 ± 1.8 mm^3) and November to December (2.0 ± 0.9 mm^3) (Fig 5A). During January to June, testes sizes were smaller. For females, it was found that small follicles of one to two millimeters in diameter were present in their ovaries throughout the year. Beginning in September, ovulation was indicated by an increased size of follicles to vitellogenesis (Fig 5B). The embryos were first observed in January (Fig 5C). However, newborns (220 mm SVL[16]) were not evident in this study whereas juvenile snakes (360 - 400 mm SVL) were first caught in June. Clutch size averaged 5.18 ± 0.37 individuals (n = 33) and increased with maternal body sizes (r^2 = 0.588; $p < 0.05$; Fig 6).

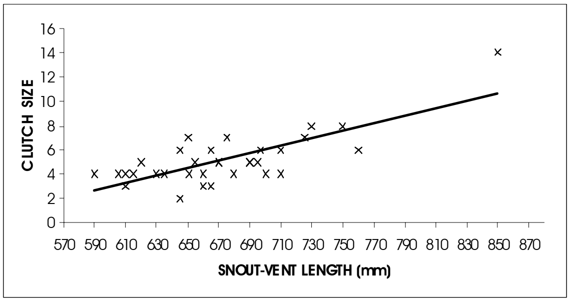

Fig 6. The relationship between snout-to-vent length (SVL) and clutch size in gravid females of *Acrochordus granulatus*. (r^2 = 0.588; $p < 0.05$).

Discussion and Conclusion

Female *A. granulatus* in Phangnga Bay had significantly larger head size, body size and longer maximum body length than males. These are restated in the positive value for the sexual size dimorphism index of 0.058. This study extends the result of earlier studies[17,18] and it is consistent with the hypotheses that many species of snakes are sexually dimorphic in adult body size with females larger than males[19,20]. Larger female body size may predict that male–male combat for females should not occur in *A. granulatus*. To explain this sexual size dimorphism (SSD), Madsen and Shine (1994)[21] suggested that adult males are smaller than females for two reasons; firstly, they grow more slowly and secondly, they have faster maturation. However, *A. granulatus* from this study showed approximately the same maturation size at 580 mm SVL in both sexes. Another possibility is that after they become mature, females may continue growing to sizes that will maximize their reproductive output. As with most vertebrates,[22] *A. granulatus* male and female juveniles follow a similar growth curve. Hence, in snake species lacking male–male combat, males seem to allocate their available energy to mate searching behavior rather than to body growth[19].

Body size differences between sexes reflect selection acting on females and males[19]. Dietary divergence has been an important selective force for the evolution of head size dimorphism in snakes. The larger head size in one sex has evolved to allow ingestion of the larger prey[23,24]. It is likely that gape size also relates to prey diameter. Rotation at the quadrate supratemporal and dentary compound joint allow the skeleton to conform more closely to prey shape[25]. This study supports the hypothesis on the relationship of maximum mouth width and head size in that the snakes with larger heads also had larger mouth widths. However, in this study food identities and food sizes from stomach contents were difficult to determine because of rapid digestive rates. Sexual dimorphism in head size may occur as a result of habitat differences[26,27]. In adult *A. arafurae*, there are also ecological differences between the sexes with small males foraging in shallow water for small fishes while larger females tend to feed on larger fishes in deeper water[28].

In this study, the male reproductive cycle was elucidated by testicular volume because the testis size has been reported to be positively related to the degree of expression of male sex hormone[29]. Examination of the testicular volume revealed that the reproductive activity in *A. granulatus* in Phangnga Bay was seasonal. Beginning in July, at the end the of monsoon season, males with large testes were observed, whereas females ovulated in September and the vitellogenic follicles were detected in November. This pattern is quite consistent with the findings of Gorman et al, (1981)[30]; a seasonal breeding of *A. granulatus* in the Philippines with snakes having the highest testosterone and testis weight in October and November. In contrast, in the Straits of Malacca *A. granulatus* reproduction has either aseasonal or loosely seasonal[1,5].

The reproductive success of female snakes in this study appear to be strongly dependent on the body size with larger females producing more offspring. Previous studies showed that larger female snakes of many species reproduce more frequently and contained more follicles than smaller females of the same species[13,14,20,31]. Thus, fecundity selection may favor the evolution of large body size in female snakes.

Acknowledgements

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