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## REPRODUCTION AND SEXUAL DICHROMATISM IN *Sitana ponticeriana* (REPTILIA: DRACONINAE: AGAMIDAE)

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### Abstract

This paper presents the results of a two-year study of the sexual dichromatism, breeding, clutch size and development in a population of *Sitana ponticeriana*, conducted at Balukhand-Konark Wildlife Sanctuary, Orissa, India. Sexual dimorphism appears as a brighter overall colouration with red and dark blue gular pouch develops as outward projection at the neck. In the breeding season, mature males have crimson-red or brick-red colour at the base of the tail. Lizards start courtship in the early morning and afternoon, depending on the ambient temperature, during the early monsoons. Vitellogenic and gravid females occur simultaneously in early and mid-summer, and lay eggs during the onset of rains. Potentially, 2-3 clutches of 6- 23 eggs (mean  $17.39 \pm 4.39$  eggs/clutch) are produced by each female each year. The smallest gravid female contained more eggs than larger ones. Eggs collected late in the reproductive season were significantly larger than those collected earlier. Freshly laid eggs are elliptical; during development, the shape, size and volume of the eggs were changed; hatchling success observed after 39 days of incubation.

**Key words:** ambient temperature, clutch size, development, Fan-throated lizard, India

### Introduction

Sexual dimorphism is a widespread feature among lizards, as in many other groups of animals (Andersson, 1994). Male and female lizards may differ in many traits, such as colouration, body shape and size, and these differences have been assigned to the effects of sexual selection (Andersson, 1994). However, other mechanisms,

such as ecological divergence (Schoener, 1967; Shine, 1991) and fecundity selection (Shine & Charnov, 1992) offer alternative explanations for sexual dimorphism (Shine, 1979). Asian agamid lizards of the genus *Draco* shows distinct morphological patterns of sexual dimorphism in body size and wing size investigated by Mori & Hikida (1992) and Shine *et al.* (1998). Many of the

lizards in the Iguanidae, Lacertidae and Agamidae families have complex display behaviors and brightly colored ornaments (Cooper & Greenberg, 1992; LeBas & Marshall, 2000). Australian agamids are no exception with widespread sexual dichromatism (Cooper & Greenberg, 1992), territoriality and highly visually oriented behaviour (Greer, 1989).

Information on the breeding biology of Indian Agamid lizards is sparse. *Calotes versicolor* breeds during the southwest monsoon from late May to October and as in other lizards, its reproductive sequence comprises renewal, breeding and post breeding phases (Shanbhag, 2002). The males are spermatogenetically active from April–September (Radder *et al.*, 2001) and gravid lizards occur from May–October (Shanbhag, 2002). In individuals with variable clutch patterns, both size and frequency of the clutch may be different depending on the proximate climatic factors, food availability, fat reserves and its maternal body size (Ballinger, 1978). Clutch size in *Sceloporus virgatus* correlates with environmental factors (Smith *et al.* 1995). Some previous studies indicate that hatchling size is not always correlated with egg size, and that proximate factors such as nesting site and breeding timing may influence this relationship (Kraemer & Bennett, 1981; Packard, 1991; Radder *et al.*, 2002). Prolonged oviductal egg retention occurs in several species of oviparous lizards (Braná *et al.*, 1991). In some of them, embryonic development of eggs continues normally during prolonged egg retention and even continues to hatching (Packard *et al.*, 1977). However, some others such as *Urosaurus ornatus* (Mathies & Andrews, 1999) arrest development until after oviposition. The garden lizard, *Calotes versicolor* retains eggs in the oviduct for as long as six months under unfavorable conditions such as a lack of proper oviposition sites or un-optimal rainfall (Shanbhag *et al.*, 2003). The maximum incubation period is 62 days; whereas, under ideal conditions the minimum incubation period is 22 days (Muthukaruppan *et al.*, 1970). Generally, reptilian eggs that incubate at high temperatures hatch earlier than those that incubate at low temperatures (Angilletta *et al.*, 2000; Deeming & Ferguson 1991). Embryonic development of *Calotes versicolor* stops below 23° C (Radder *et al.*, 2002; Shanbhag *et al.*, 2003). Its eggs have a flexible eggshell and increase in volume after oviposition, with an encroachment in embryonic growth primarily due to infused moisture (Radder *et al.*, 2002).

The fan-throated lizard, *Sitana ponticeriana* is a medium-sized, ground-dwelling agamid scattered throughout open xeric patches of forest in India (Pal *et al.*, 2010). There are only few studies on the biology of *Sitana ponticeriana* (Pal *et al.*, 2007; Rajkumar & Shanbhag, 2003, 2004; Rath & Pal, 2007) and its reproductive biology remains subject to speculation because of small sample sizes over limited periods of time. Here, we report on sexual dimorphism, breeding, clutch size and development of *Sitana ponticeriana* over a two-year period.

### Materials and methods

**Study area:** This study took place on the Balukhand-Konark Wildlife Sanctuary (BKWS) in the District of Puri, Orissa, India (19°48' to 19°54' N, 85°52' to 86°14' E) (Pal *et al.*, 2010a). The Sanctuary is a 71.7 km<sup>2</sup> area established on a sandy tract along the coast between Puri and Konark. The canopy composition includes mainly *Casuarinas* sp., Tamarind (*Tamarindus indica*), *Anacardium* sp., Karanja (*Pongamia glabra*), Polanga (*Calophyllum inophyllum*), Neem (*Azadirachta indica*), *Eucalyptus* sp., and *Acacia* sp. The understory shrubs include *Pandanus* sp., *Adhatoda* (*Adhatoda vasica*), and various horticultural plantings. The lizard fauna includes *Lygosoma punctata*, *Eutropis bibroni*, *E. macularia* and *Calotes versicolor*. The maximum air temperature is 40°C during May-June. Maximum surface soil temperature ranges from 40°C in the shade to over 45°C in areas with full sun. Minimum winter temperature is 10°C.

**Maintenance in the laboratory:** We collected lizards by hand from October 2001 through September 2003 and after an acclimatization period of about 2-7 days in the laboratory; we sexed the specimens by examining the gonads and secondary sexual characteristics. We categorized the lizards as juveniles (SVL = 15-26mm), immature (SVL = 27-35mm) and mature adults (SVL ≥ 36 mm above) including gravid females (Pal *et al.*, 2010a,b). We fed the lizards by Isoptera, Hymenoptera, Orthoptera, Acrididae etc. (Pal *et al.*, 2007) and provided water *ad-libidum* each day. We collected gravid females of *S. ponticeriana* during the months of May-August (2001 and 2003) exclusively for determining clutch size. We recorded snout-vent length (SVL), tail-length (TL) and total length (TOL) for each lizard. We confirmed oviposition following the methods adopted by Shanbhag *et al.* (2003). Each terrarium contained a few potted plants, dry sticks and leaves, hiding sites, water and a substratum of sandy soil from the capture site. Gravid females were found

from May to October, consequently, we classified them as early, mid- and late breeders depending on the captured date. After egg laying, we incubated the eggs in moist sandy soil at ambient temperature  $\sim 35 \pm 2^\circ\text{C}$ ). We did not consider second or third clutches in our study because these developed in the female's body while she was in captivity. After the first clutch, we returned the adult lizards to the sites where we originally captured them. Eggs hatched after 39 days of incubation and  $>10\%$  of eggs hatched for each clutch. We recorded the SVL, TL and TOL of the hatchlings and then released them into the Sanctuary after 2-3 days.

**Statistical analysis:** We used Chi-square to compare sexual dimorphism and reproductive status between the two years in our study. We identified the reproductive characteristics between early and late in the breeding season with regression analysis. Student's *t*-tests were used when the covariant was not obvious. We presented all of the averages  $\pm$  SE unless otherwise indicated. We used  $\alpha = 0.01$  for decision theory in all statistical tests.

## Results

**Sexual dimorphism and dichromatism:** Both sexes were olive-brown above and whitish below neck. The dorsum of body is dark-brown with black-edged diamond-shaped rhomboidal patches in both sexes. The gular pouches of males turned brilliant red and dark blue during the breeding season. Males unfolded and folded their pouches several times to entice females during courtship. During the breeding season the tail of mature males are crimson-red or brick-red; whereas, the females have a brown coloured tail. Immature adult males show a white flap-like structure below the neck. However, the mean of SVL, TL and TOL of 256 mature males were  $43.51\text{mm} \pm 5.96$ ,  $90.22 \text{ mm} \pm 12.44$  and  $133.63 \text{ mm} \pm 17.95$ , respectively. Similarly, the mean of SVL, TL, and TOL of 156 mature females were  $43.96 \text{ mm} \pm 6.82$ ,  $84.26 \text{ mm} \pm 12.44$  and  $128.21 \text{ mm} \pm 17.98$ , respectively. The *t*-values between SVL, TL and TOL of the males and females were 0.69, 4.69 and 2.95 respectively and were significant at 1 % level (Table 1). The regression equation of males and the females were  $\text{TL} = 1.637 \text{ SVL} + 0.0072$  and  $\text{TOL} = 2.5793 \text{ SVL} + 0.0248$  and  $\text{TL} = 1.7392 \text{ SVL} + 0.8364$  and  $\text{TOL} = 2.7864 \text{ SVL} + 0.6843$  respectively (Fig. 1a,b,c,d)

**Breeding:** *Sitana ponticeriana* breed from pre-monsoon through post-monsoon periods (May to September). The peak breeding season was from June through July, but occasionally, an early or late

monsoon stimulated breeding as early as the end of April or as late as September, was recorded. This species required some form of weather conditions such as minimum amount of rainfall and ambient temperature should be  $< 40^\circ\text{C}$  before breeding. The maximum number of mature males and the gravid females appeared in April through July. During an early monsoon several mating pairs of the species were active in the field from April through August, but the breeding males were more active than the females. Lizards start courtship in the early morning and afternoon, depending on the ambient temperature, and high ambient temperatures at midday appeared to preclude mating during that time. Interestingly, sometimes mating has been observed throughout the day, on cloudy days.

**Clutch size:** We collected all the adult lizards ( $n = 86$ ) during October 2001 through September 2003. Lizards were reproductively active as obvious by the presence of either vitellogenic follicles or oviductal eggs or both. The SVL of the smallest female with oviductal eggs was 37 mm, and that of the largest, 58 mm. Clutch size averaged  $17.39 \pm 4.39$  (Table 2) and varied from 6-23 eggs (Fig. 2). Maternal SVL and clutch size was not positively correlated. There was no significant relationship between egg mass and clutch size. Early clutches were larger than those from later clutches.

**Development:** Lizards exhibit prolonged oviductal egg retention when oviposition sites, certain proximate like high ambient temperature and lack of rain falls affect egg-laying behaviour. The egg retention was systematically studied under laboratory condition. The lizards held eggs in their oviducts for about three to four weeks before oviposition. However, their reproductive output was dependent on the climatic factors (e.g., rainfall) that they normally experienced in the wild. We observed in our terrarium, that oviposition was not fixed in a particular place rather it was here and there. It took 10-20 minutes to complete and some lizards (60%) even took 30-45 minutes (2%) even took 30-45 minutes. We found 6-8 eggs in the terrarium that we immediately measured (Table 2), and then placed them under incubation. The eggs were oval or elliptical with flexible shells and coloured white. We measured the length and breadth of those eggs that had not died after 25-32 days of oviposition. Egg volumes increased with embryonic growth up to 32 days. We removed some eggs after 32 days of incubation and observed the growth stage of the lizard. Successful hatching occurred after 39 days of incubation.

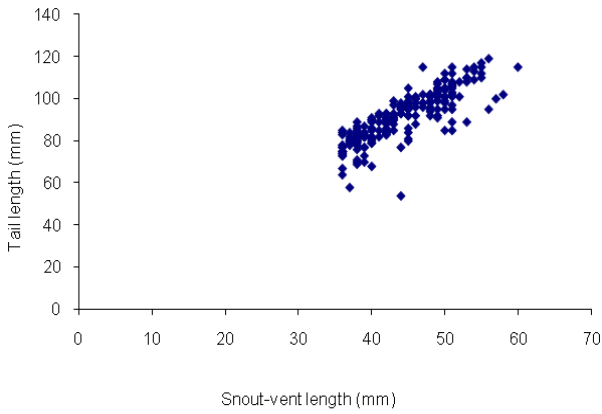


Fig. 1a: (A) Plot of TL against SVL of mature male *Sitana ponticeriana* at BKWS

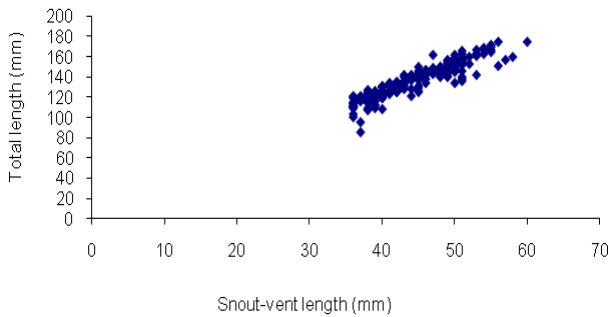


Fig. 1b: Regression curves of TOL against SVL of mature male *Sitana ponticeriana* at BKWS

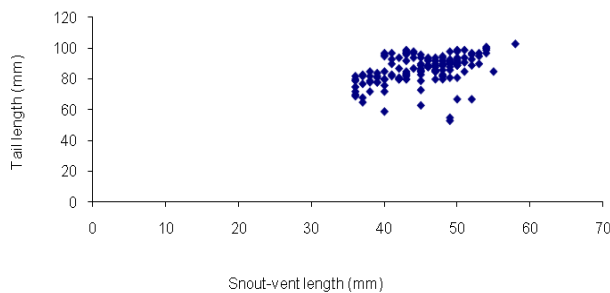


Fig. 1c: Regression curves of TL against SVL of mature female *Sitana ponticeriana* at BKWS

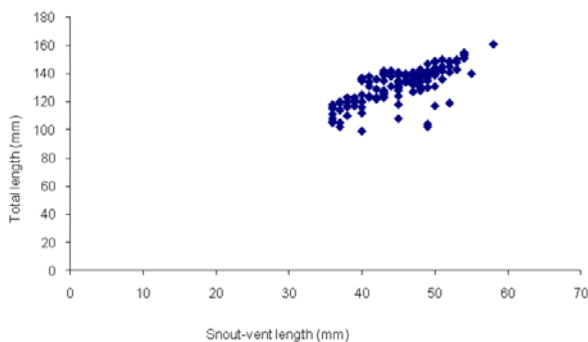


Fig. 1d: Regression curves of TOL against SVL of mature female *Sitana ponticeriana* at BKWS

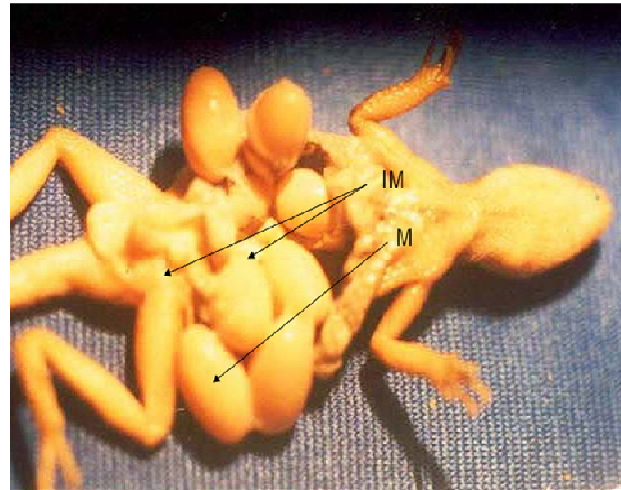


Fig. 2: Mature (M) and immature (IM) eggs of dissected gravid *Sitana ponticeriana* (SVL: 49mm) from BKWS

### Discussion

The evidence for female choice in lizards is at best equivocal (Olsson & Madsen, 1998) and many taxa display remarkable sexual dichromatism with males of many species having ornaments such as rostral horns, dewlaps, gular sacs and crests. This is true of agamids, chamaeleons and their New World sister group, the iguanids. Colour patches may serve as badges of status among males and can affect the outcome of male contests in some species (Olsson & Madsen, 1998). Here, *Sitana ponticeriana* showed a distinctive red and dark blue gular pouch developed under the neck of male that they used to attract female lizards during the breeding season using the crimson red colour at the base of tail. For instance, there is evidence that size dimorphism may be associated with territoriality, social polygyny and male–male competition in lizards (Olsson & Madsen, 1998; Stamps, 1983). Size dimorphism in lizards can reflect ecological resource partitioning (Magurran, 1998; Perez-Mellado & de la Riva, 1993; Shine, 1979). Our study suggests a negative association between size dimorphism that may reflect mechanisms other than intersexual selection (female choice) in *Sitana ponticeriana*.

Many previous publications reported the clutch sizes of *Calotes versicolor* (Erdelen, 1986; Rout & Ghosh, 1984). The present study on the clutch size of *S. ponticeriana* confirms some previous reports, but contradicts others. Clutch size was larger in our study than in previous reports. Subba Rao & Rajabai (1972) reported that these lizards produce 8–13 eggs with a mean value of 11.2 eggs/clutch ( $n = 10$  clutches, SVL range 36–52 mm), but Tikader & Sharma (1992) reported 11–14 eggs/clutch.

According to Rajkumar & Shanbhag (2003) the clutch size varies from 7-19 with a mean value of 15.47. In our study, clutch size varied from 6-23 eggs per clutch, but a mean value of 17.39 (n = 88 clutches) was noted. The smaller clutch sizes reported in the previous studies may stem from smaller females, geographic variation, or other environmental factors that regulate clutch size. However, clutch size did not differ between the two years of our study.

Clutch size of *S. ponticeriana* was not increased with maternal SVL, and there was no evidence of any relationship between maternal SVL and egg size. Further, the lack of an inverse correlation between clutch size and egg size suggests that there was no trade-off between these two reproductive traits, implying that there is optimization of egg size. This species produces at least two to three clutches in a breeding season. The early clutches are larger than the later ones as reported in other species of lizards (Shanbhag, 2002, 2003). Decrease in clutch size results from a trade-off between egg size and clutch size (i.e., larger eggs, but few in number) where food availability and modes of energy allocation exist in several species of lizards (Shanbhag, 2003).

The present study on development of *S. ponticeriana* occurred over 30-39 days in captivity. This species shows polyautochory because it ovulates many eggs simultaneously from both of its ovaries as also seen in garden lizard (Radder *et al.*, 2001). Eggs of *S. ponticeriana* increase in volume with embryonic growth following oviposition. The increase in the size of eggs is due to infused moisture (Shanbhag *et al.*, 2003). Further, we speculate that arresting embryonic growth also lessens physiological demands for oxygen and moisture needed by the growing embryo. Shanbhag *et al.* (2003) demonstrated that embryonic diapause during prolonged egg retention occurred in *Calotes versicolor* when its body temperature is lowered by 3-5°C.

Our investigation supports the ambient temperature and rainfall, both which influence the amount of food that increasing energy available for reproduction of this species. Further, low temperature, cloudy skies and wet conditions may interfere with lizard thermal ecology but interestingly, these characters strengthening the reproductive behaviour also resulting in complex interactions with life history of this subtropical lizard.

**Table 1:** Summary of the sexual dimorphism traits in mature male (N=256) and female (N=156) of *Sitana ponticeriana* at BKWS, Orissa, India from September 2001-October 2003.

	SVL ± SD (mm)	TL ± SD (mm)	TOL ± SD (mm)
Male	43.51mm ± 5.9	90.22 ± 12.44	133.63 ± 17.95
Female	43.96 ± 6.82	84.26 ± 12.44	128.21 ± 17.98
t-Test#	0.69	4.69	2.95

#  $\alpha = 0.01$

**Table 2:** Summary of the recorded traits in *Sitana ponticeriana* at BKWS, Orissa, India from September 2001-October 2003

Variable	Mean ± SD	Range
Maternal SVL (mm)	46.68 ± 6.38	37-58
Clutch size (n)	17.39 ± 4.39	6-23
Egg mass (mg)	0.11 ± 0.01	0.08-0.13
Egg length (mm)	9.87 ± 1.33	8-11
Egg width (mm)	5.04 ± 0.75	4-6
Offspring SVL (mm)	13.8 ± 2.47	13-15
Offspring TL (mm)	26.6 ± 1.06	25-28
Offspring TOL (mm)	40.4 ± 0.89	38-43

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