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Female Reproductive Strategy of Common Water Monitor (Varanus salvator) from Riau Region

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Abstract

Reproductive strategy of a species is overall reproductive pattern to improve its fertilization chances and offspring's survival rate. Common water monitor (*Varanus salvator* ssp.) is the second largest lizard and has wide distribution area in Oriental. Hence, it is predicted to have different reproductive strategy which is caused by environmental. In this study, we report the direct observation on females *V. salvator* reproduction in Riau region, Indonesia. The main objectives of this study were to determine the reproductive strategy of females *V. salvator* with regard to maturity at size, clutch size, egg size, reproductive season and reproductive potential. Twelve *V. salvator* from six locations, namely Bengkalis Island, Pekanbaru, Siak, Mendol Island, Kundur Island, and Batam Island were dissected to study their reproductive strategy. Comparative data on lizards in the other tropical zones from previous studies were reviewed. Our result shows that the presence of an ovarian follicular hierarchy is a characteristic feature of this species. This lizard is categorized as mature at smaller proportion of maximum size, has small clutch size and produce multiple-clutching.

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Therefore, the reproductive potency of *V. salvator* may be high. These reproductive strategies, except for the reproduction season are similar with the lizards from Sumatra, Hainan and India.

Keywords: follicular hierarchy; multiple clutches; reproductive potential; reproductive season.

1. Introduction

Reproductive strategy of a species is overall reproduction pattern to improve its fertilization chances and offspring's survival rate. Variation of reproductive strategy is usually associated with reproductive traits [1] such as ovarian organization, maturity at size, clutch size, egg size and reproductive season. The different strategies have been well documented in lizard [2-5] which have wide area distribution as *Sceloporus variabilis* from Atlapexco, San Pablo Tetlapayac, Santa Catarina, Monte Pio, and Bastonal in Mexico [6, 7]. Reproductive variation in each population could be the result of both historical (phylogenetic; e.g., reproductive mode) or environmental (e.g. temperature, food availability) factors [7].

Common water monitor (*Varanus salvator* ssp.) is the second largest lizard and is widely distributed from Northern India, Sri Lanka, Andaman Islands, Burma (Myanmar), Thailand, Malaysian, Peninsula, Indonesia, Cambodia, Laos, Vietnam, and southern China (Hainan) [8]. In Indonesia, this species is found in Sumatra and its small off-shore islands (Nias, Siberut, Simalur, Bangka, and Belitung), Borneo, Java and Lesser Sunda, Northern Celebes and Obi Island [9-12]. Due to its wide distribution area with different geological history, the lizard is supposed to have reproductive variation. However, we show that local environment factor plays more significant role to cause the invariant of *V. salvator* reproductive variation.

Here we report the direct observations on females *V. salvator* reproduction in Riau region, Indonesia. The main objectives of this study were to determine reproductive strategy of *V. salvator* with regards to maturity at size, clutch size, egg size, estimation reproductive potential and reproductive season.

2. Materials and Methods

2.1. Ethic statement

We obtained the permission to collect *V. salvator* of Riau region from Balai Besar Konservasi Sumber Daya Alam (BBKSDA) Riau. The study was performed in compliance with the protocols approved by the Animal Care and Use Committee of Bogor Agricultural University (IPB), Bogor, Indonesia (ACUC No. 13-2013 IPB).

2.2. Data collection

The female common water monitors in Riau region, e.g. Bengkalis Island (n=2), Pekanbaru (n=2), Siak (n=2), Mendol Island (n=1), Kundur Island (n=3), and Batam Island (n=2) were caught from natural habitat from August until December 2013. We recorded the snout-vent length (SVL) and tail length. These animals were anesthetized using ketamine 50 mg/kg BB prior to dissection. Dissection on ventral median body, start at perineum to sternum. The several sterna were cut to reach heart. Exsanguination was done by injecting a cannula which was connected to a hose containing *physiological saline* (0.90% w/v of NaCl, 308 mOsm/L or 9.0 g per liter) at the left ventricle and cut of right atrium to the irrigation process until the liquid out of the right atrium looks clear. Their reproductive organ was isolated and preserved using FAAC (formaldehyde 4%, acetic acid 5%, calcium chloride 1.3%) by perfusion when the atrium still pulse. We observed situs viscerum to see structure, form and landmark of organ reproductive to the other organ. The width and length of the oviducts and ovary were measured.

We observed and classified the condition of the oviducts (CO) into three categories: smooth, striated and convoluted [13]. Smooth oviducts were straight, without visible marks of thickening or stretching. Striated oviducts were straight with noticeable transverse striation along the entire oviduct. Convoluted oviducts were convoluted throughout their entire length with thicken walls.

Ovarian development of *V. salvator* was determined based on the ovary condition, which was evaluated by assigning diameters of follicles in several classes (Table 1). The sizes of ovarian follicles / eggs were measured to the nearest 0.1 mm using a vernier caliper. The follicles diameters were determined by averaging major and minor length. The condition of the ovary was evaluated by classifying the various phases of follicle into six classes [sensu 14] and then the follicles were scored in these classes. We also examined the presence of ovulation scars (corpora lutea and corpora albicantia) in the ovary from the previous clutches.

Table 1: Classes of ovarian follicles and oviductal eggs in *V. salvator* base on size [sensu 14]

Follicles or eggs group	Diameter of follicles or eggs	Note
I	<= 1.00 mm	undeveloped follicles
II	1.01-3.00 mm	early growth of follicles
III	3.01-8.00 mm	hydration and initial vitellogenesis
IV	8.01-23.91 mm	active vitellogenesis
V	>=23.92 mm	maturation, immediately preceding ovulation
VI	>=26.57 mm	oviductal eggs: the presence of eggs in the oviducts

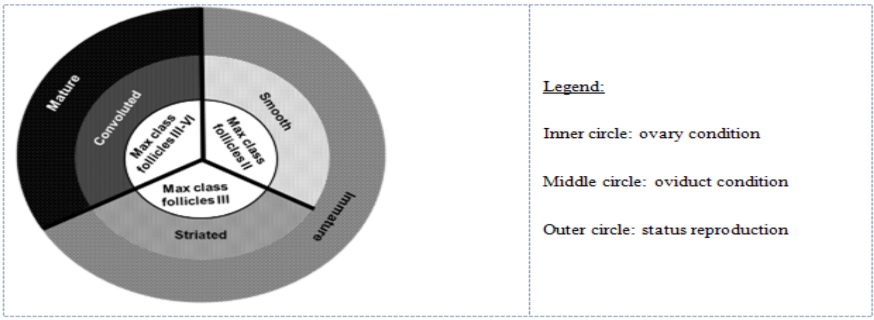

Figure 1: Categories of status reproduction in *V. salvator* [13, 14]

We categorized the status reproduction as immature and mature using ovary and oviduct condition data, (Figure 1). The maturity size of *V. salvator* was determined from the overlap in the SVL of the largest specimen that was judged to be immature and the smallest reproductive specimen. The reproductive potential was estimated from the clutch size and the number of clutch/year. All of data was analyzed descriptively and was presented in table and figure.

3. Result

V. salvator has internal fertilization strategy. As an adaptation of internal fertilization strategy, the female reproductive organs consist of a pair oviduct, ovary and hemiclitoris. They are laid under adipose tissue and digestive organs in the abdominal cavity (Figure 2).

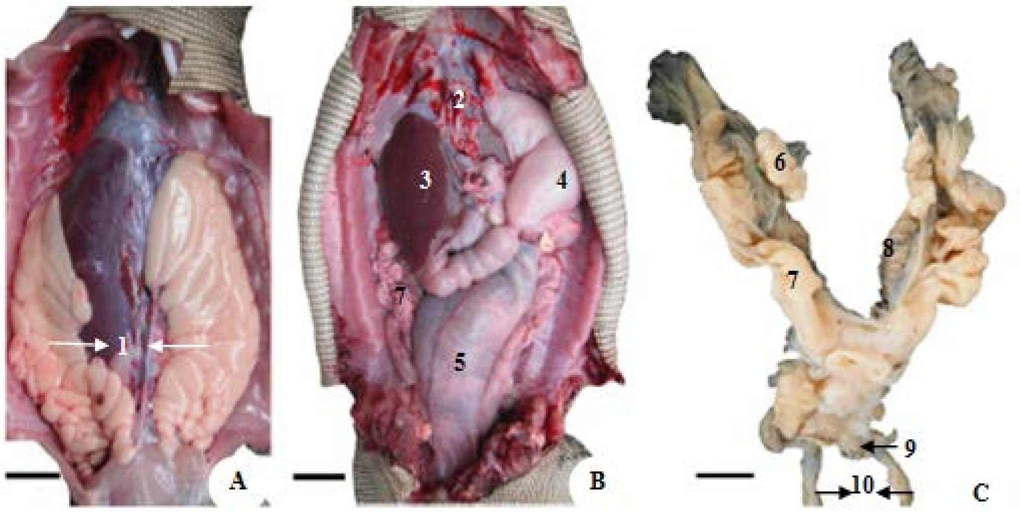

Figure 2: Ventral view of dissected female *V. salvator*. A. Fat pad has not been removed. B. Dissected female specimen exposing the general viscera organs. C. Urogenital tractus of female *V. salvator* . 1. Fat pad, 2. Heart, 3. Liver, 4. Stomach, 5. Large intestine, 6. Ovarium, 7. Oviduct, 8. Kidney, 9. Hemiclitoris,10. Musculus retractor magnus. Scale bar = 2 cm

The smooth oviducts were not yet differentiated in juvenile individual (Figure 3A). Sub adult and adult

V. salvator had striated and convoluted oviduct, respectively (Figure 3B & 3C). Gravid female had stretch oviducts (Figure 3D). The right and left oviducts of *V. salvator* which was gravid contained 4 and 3 eggs, respectively.

The oviducts of water monitor lizard were structurally pair, consisted of an anterior infundibulum tube, a middle uterus and a posterior vagina. The dorsal oviduct was suspended by mesentery which was continuous with the peritoneum and binds the vaginal and uterine segments to dorsolateral. The right oviducts lied more anterior than their counterparts on the left. The ventral oviduct was bound by a smooth muscle band which extended from the vagina to the posterior border of the infundibular ostium. The oviducts of juvenile were not yet differentiated (Figure 3A). The sub adult individual and the adult had striated and convoluted oviduct, respectively (Figure 3B & 3C). The gravid oviduct was stretch (Figure 3D). The right and left oviducts of *V. salvator* which was gravid contained 4 and 3 eggs, respectively.

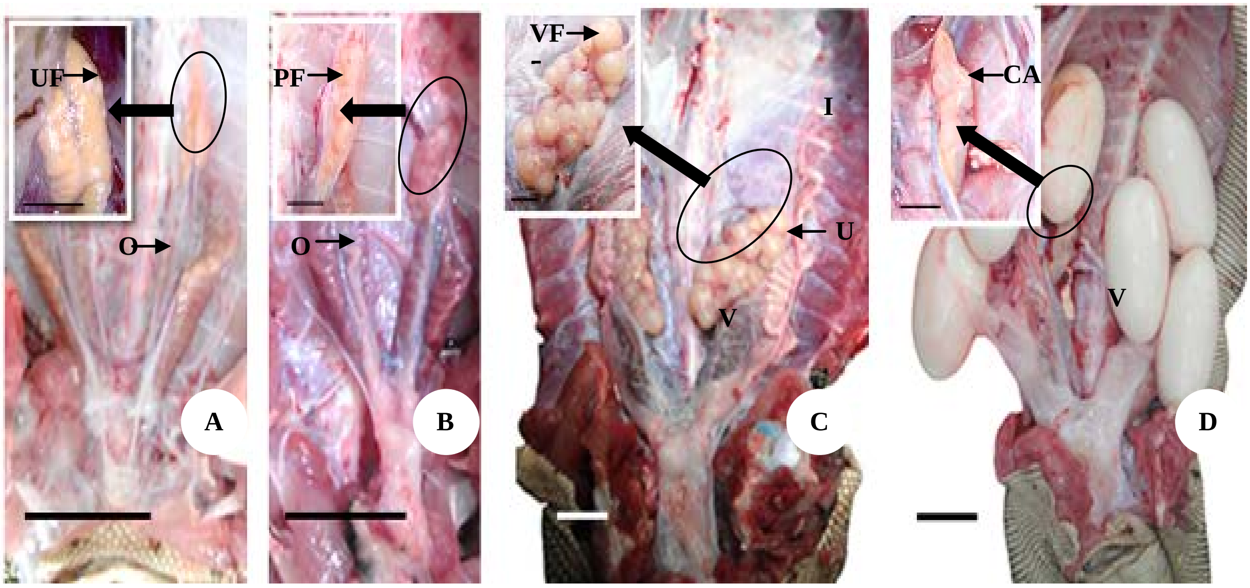

Figure 3: Oviduct types (outset) and ovary condition (inset) of *V. salvator* in ventral view. The digestive tract was removed to reveal the symmetry of the reproductive tract; the head was oriented towards the anterior of the photograph. A. Smooth oviduct. B. Striated oviduct. C. Convoluted oviduct of non-gravid female. D. Stretched oviduct of the gravid female. O: oviduct; I: Infundibulum; U: Uterus; V: Vagina. UF: Undeveloped follicle; PF: Previtellogenic follicle; VF: Vitellogenic follicle; CA: Corpus albicantia. Oviduct scale bar: A, B, C, and D = 2.0 cm. Ovary scale bar: A = 0.5 cm; B, C and D = 1.0 cm

Ovaries of *V. salvator* were located above the kidney and were suspended by ligaments mesovarium in the peritoneal cavity. In general, the shape of the ovary depends on the stage of development and reproduction activities. In juvenile, ovaries were compact, fore-and aft, has bright peach background and transparent with white small granule. The texture of the small granule was hardly visible to the naked eye due to the high content of undeveloped follicles (Figure 3A). Ovary of sub adult (Figure 3B) had elongated form. Pre-ovulation ovaries of adult individual (Figure 3C) were more swollen than ovary of sub adult because they had vitellogenic follicles. The redness and transparency of vitellogenic follicles were caused by a high vascularity. Vitellogenic follicles indicated the individual reproduction actively. The gravid of *V. salvator* had post ovulation ovary regressed with a maximum size of less than 8 mm follicles (Figure 3D). Therefore the wide stretch oviduct and follicular activity in *V. salvator* appears to be synchronous (Table 2) except in gravid of *V. salvator.*

We assume that each follicle class represents a discrete group. Each follicle is not always able to evolve to the next stage because there are follicles that will become atresia or atrophy (Figure 4). Atrophy of some portion of follicles in certain class seems certain, because the number that certain class follicles is always more than the next class. Atrophy in vitellogenic is smaller than it in previtellogenic follicles.

Table 2: Size of *V. salvator* oviduct and ovary (mm)

Stage	Oviduct		Ovary	
	Length	Width	Length	Width
Juvenile (n=1)	73.00	1.97	17.00	2.76
Sub adult (n=7)	(*x* = 87.90)	(*x* = 4.24)	(*x* = 27.13)	(*x* = 8.85)
	63.50-115.5	3.65-6.05	13.9-33.00	5.20-9.15
Adult non gravid	(*x* = 161.00)	(*x* = 10.56)	(*x* = 48.80)	(*x* = 15 .00)
(n=3)	116.00-215.00	8.32-11.88	35.25-67.50	9.25-15.85
Adult gravid (n=1)	624.00	stretched	41.00	12.50

Note: n: the number of specimen, *x* = mean value.

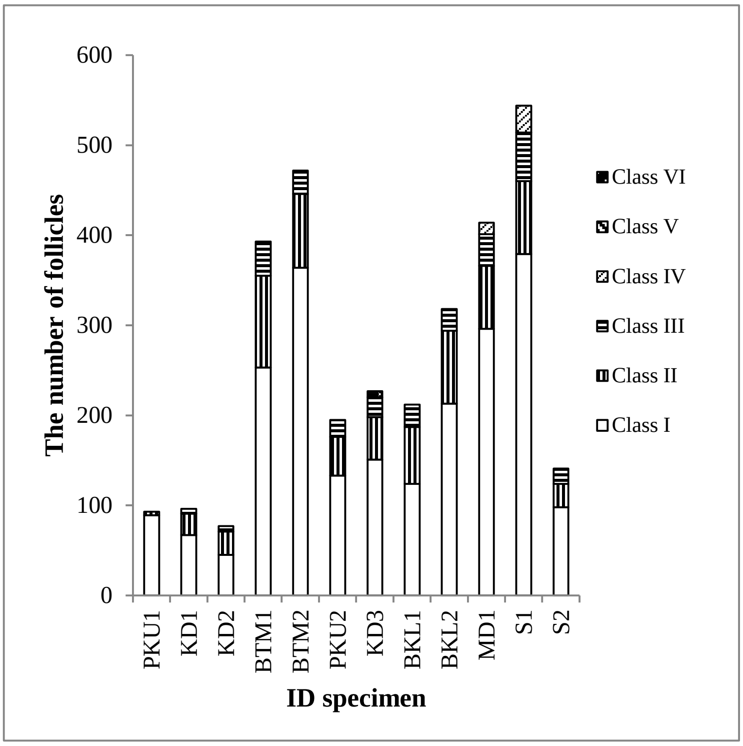

Figure 4: The number of follicles base on diameter class follicles in each water monitor. PKU: Pekanbaru, KD; Kundur, BTM: Batam, BKL: Bengkalis, MD: Mendol, S: Siak.

V. salvator from Riau is assumed to produce multiple clutches each year as also shown from the lizard in northern and southern Sumatra [15, 16] and northern India [17]. It is shown by the ovarian organization in reproductive active female which contains previtellogenic and vitellogenic follicles. The previtellogenic follicles are predicted to evolve to the next egg clutches.

Combining the sexual maturity of female Riau's *V. salvator* (n=4) yields an average SVL of 59.10 ± a standard deviation of 8.08 cm and a range of 49.30 – 68.50 cm. The SVL of female with oviducal eggs is 49.30 cm (Table 3). Thus, estimate size at maturity of this species approximately 50.00 cm even if they do not possess vitellogenic follicles or oviductal egg. The result was relatively similar with female *V. salvator* from Palembang and Medan which mature with SVL of 47.00 and 48.50 cm, respectively [15, 16]. Based on proportion of maximum SVL at maturation with maximum attainable SVL, lizards with larger SVL tend to have smaller SVL maturity at size. The proportion of the biggest and the smallest lizard are 30.00% for *V. komodoensis* [18] and 74.60% for *V. bervicauda,* respectively [19, 20]. Therefore, *V. salvator* which has 42.74% proportion matures at small size [15, 20] and it is also shown by the other squamates [21].

Table 3: Reproductive status of Riau water monitor lizard

ID	SVL (cm)	Ovary condition (maximum diameter class of follicles)	Oviduct condition	Reproductive status
Pekanbaru1	37.70	II	smooth	immature
Kundur1	27.90	III	striated	immature
Kundur2	41.20	III	striated	immature
Batam1	47.80	III	striated	immature
Batam2	48.70	III	striated	immature
Pekanbaru2	49.00	III	striated	immature
Kundur3	**49.30**	**VI**	**convoluted**	**mature**
Bengkalis1	49.50	III	striated	immature
Bengkalis2	50.00	III	striated	immature
Mendo1	56.90	IV	convoluted	mature
Siak1	61.70	IV	convoluted	mature
Siak2	68.50	III	convoluted	mature

The clutch size of *V. salvator* in Riau region was seven eggs (n=1) and it falls within the range 5-22 of southern Sumatra [15] and 7-17 of northern India [17]. However, the size was low compared to Yu's study in China and Smith's study in Siam which reached 10-23 and 15-30, respectively. [22, 23]. Our data do not permit us to suggest a correlation between body size (SVL) and clutch size based on one sample. However, based on the previous studies, body size was positively correlated with clutch size [15, 16, 23, 24]. *V. salvator* is predicted to have high reproductive potential because it matures at small size, has small clutch size and produce multiple-clutching.

Egg size of *V. salvator* showed no distinct variation across the geographic range. The average dimension of the eggs in the oviduct in our observation was 66.66 ± 2.66 x 30.40 ± 2.12 mm. The egg size in our oviductal is lower than Sumatran oviductal eggs (75.00 x 40.00 mm) [15] because the Sumatran eggs are already to be oviposited. The average of oviductal eggs size are slightly lower than freshly –laid egg as 74.02 ± 0.61 x 39.57 ± 0.55 mm in India [17] and 74.90 ± 1.30 x 42.30 ± 0.50 mm in Hainan [23]. It would be expected since the eggs may gain water rapidly after deposition [25].

The reproductive season of *V. salvator* is determined by local climate and their geographic distribution. Our study can not to estimate reproductive season as the number of *V. salvator* from Riau is small. However, Riau region climate has minimum precipitation of 60 mm and included equatorial rainforest. Then, reproductive season of the species is supposed to occur throughout the year. The result is consistent Sumatran lizards from rain forest shows continuous reproductive patterns [15]. Lizard reproduction, in tropical region with pronounced wet and dry season, tends to be concentrated in the months with high rainfall [22, 26]. West Bengal, India; eastern and southern (peninsular) Thailand have annual precipitation of ≥ 25 (100-P_{min}) mm [27]. Hence, the breeding season in India and Thailand occurs from April-July [22, 28, 29]. While in Ledong, Hainan with P_{min} < 60 mm in winter [27], the breeding season is stretching from mid-June and mid-September [23].

4. Discussion

We showed that the predicted of having different reproductive strategy in *V. salvator* is not entirely proven. This lizard shows similar multiple-clutching, maturity at size, egg size and reproductive potential estimation across its geographic range. It may be caused by the stability climatic of regime where the availability of food is always abundance. Additionally, the diet range of *V. salvator* is extremely broad such as fish, frog, snake, marsh crab, snail-eating turtle, duck, water hen, rat, cat, dog, carcass, and human food leftovers [30, 31].

Contrary, reproductive season of *V. salvator* shows variation across their geographic distribution [22, 23, 28, 29]. The lizard distribution encompasses climatic diversity on both sides of equator [27] especially at the first heavy rains within a long dry period. It may trigger hormonal of female and male of *V. salvator* to reproduce [28].

Presumably, the reproductive strategy may contribute to international conservation about the long term availability of this species. This study also gives information for captive breeder that *V. salvator* has high reproductive potency and a high range of food. Moreover, this study also helpful to provide basic information of conservation management after the advance study about monitoring population and reproduction of *V. salvator* from harvested sites has been done.

Due to the limited number of samples, we can only suggest some reproductive variables such as reproductive season and the number of clutch annually. To ensure the information is required further research by the numbers of adult female are sufficient and time observations are carried out annually. Reproductive strategy for males is also required to study for completing the reproductive of *V. salvator*.

5. Conclusions

Reproductive strategy of female *V. salvator* from Riau can be described as mature at smaller proportion of maximum size, has small clutch size, multiple-clutching and reproductive season occur throughout the year. *V. salvator* reproductive strategy has less variation across the range of their geographic distribution.

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