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***Research Article***

# THE OOGENIC CYCLE OF THE DICE SNAKE, *NATRIX TESSELLATA TESSELLATA* (SERPENTES: COLUBRIDAE) IN NORTHERN IRAN

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**ABSTRACT**
The dice snake, *Natrix tessellata tessellata* belongs to the family Colubridae, is an oviparous snake lives mainly in most zones of Iran except eastern south. In this research, the oogenic cycle of *Natrix tessellata*, was studied in rice lands and rivers of Sari County in Mazandaran Province, northern Iran. Sampling took place periodically every 15 days during the activity period of this species, from 5 April to 5 November 2012. In total, 63 adult females were captured by hand and net. Ovaries and oviducts were removed and processed for histological and morphometrical studies. Oogenesis start at early April, reaching its peak in early and mid June, and ends between early to late July. Oviposition occurred from late June to early August. Folicle number varies between 3-39 in each ovary. The follicle diameter varies between 1.18-43.35 mm. The mean diameter of follicular layer and tunica albuginea varies between 40-141 and 13-31.5 micron, respectively. The numbers of oviductal eggs are 1-12 in each oviduct. In Iran, the oogenic cycle of *N. tessellata* is seasonal and alternate from April to July.

***Keywords:*** *Natrix Tessellata Tessellata, Colubridae, Oogenesis, Reproduction, Iran*

## INTRODUCTION

The dice snake (tessellated water snake), *Natrix tessellata tessellata* (Laurenti, 1768) is a non-venomous oviparous snake of the family Colubridae lives mainly in most zones of Iran except eastern south (Figure 1). The only recognized subspecies of this species in Iran is *Natrix tessellata tessellate* (Rastegar-Pouyani *et al.*, 2008). The dice snakes are semi-aquatic snakes, live in moist areas usually very close to water in the form of streams, rivers and ponds, and feed mainly with amphibians and fishes. They are diurnal and bask in the morning sun to raise their body temperatures. When disturbed, they usually swim away at high speed; they usually avoid diving, opting to stay on the water surface. Their preferred terrestrial habitats appear to be open woodland and edge such as field margins and woodland borders, as these may offer adequate refuge while still affording ample opportunity for thermoregulation through basking (Latifi, 1991). This species is abundant in northern Iran. The other semi-aquatic snake in this region is grass snake, *Natrix natrix* that was studied in recent years (Faghiri *et al.*, 2011; Shiravi *et al.*, 2012; Hojati *et al.*, 2013). The main studies on the identification of snakes of Iran were performed by Latifi and Farzanpey (Latifi, 1991; Farzanpey, 1990); however, there wasn't any specific study on the oogenic cycle of this species. This research was conducted to study the oogenic cycle of *N. tessellata tessellata* in Mazandaran, a northern province of Iran.

## MATERIALS AND METHODS

### *Study Area*

The study locality was Sari county (36°32 N 54°7 E), in Mazandaran province in northern Iran, located on the southern coast of the Caspian Sea. Four stations near Sari city were selected, including: Ab-Bandansar fish training pond; Shahid Zaree Forest; Sari rice fields; and Tajan River (Figures 2 and 3). The climate of this area is wet and temperate (the mean temperatures of the coldest and warmest seasons were 1.6 and 22.5°C, respectively), and the most dominant plants are grassy species belonging to the family Gramineae.

***Sampling*:** Sampling took place periodically (every 15 days) between April and October 2012. Sampling time was during daylight from 8 a.m. to 4 p.m., especially around noon. We captured 92 specimens by hand and fishing net which 63 specimens were female.

**Figure1: *Natrix tessellata tessellata* in Mazandaran Province, Iran**

**Figure 2: Ab-Bandansar fish training pond in Mazandaran Province, Iran**

***Experimental Methods*:** The specimens were fixed and preserved in 96% ethanol. Body length (SVL), tail length (LCD) and body weight (W) were measured. Gonads, once removed and ROD (diameter of right ovary), LOD (diameter of left ovary), ROW (weight of right ovary), LOW (weight of left ovary), ROV (volume of right ovary), LOV (volume of left ovary), RFN (number of right follicles), LFN (number of left follicles), MaxRF (maximum diameter of right follicles), MaxLF (maximum diameter of left follicles), MinRF (minimum diameter of right follicles), MinLF (minimum diameter of left follicles), MRF (mean diameter of right follicles), MLF (mean diameter of left follicles), GI (Gonadal index =

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gonadal wt/body wt × 100), MDRFL (mean diameter of follicular layer of right follicles) and MDLFL (mean diameter of follicular layer of left follicles) were measured. Length, width and diameter were measured by dial caliper with an accuracy of 0.02 mm. Weight was measured by a scale with an accuracy of 0.001 g. After fixing the ovaries in 10% formalin, tissues were dehydrated, cleared in xylene, infiltrated with paraffin, embedded and sectioned and stained with haematoxylin and eosin following standard histological protocols. The sections were studied by light microscopy at 100× and 400× magnification. Photographs were prepared by digital camera. Data were analyzed by SPSS 17 software, ANOVA and paired t-test ($P < 0.01$).

**Figure 3: Sari rice fields in Mazandaran Province, Iran**

**RESULTS AND DICSCUSSION**

The tessellated snake in the study area hibernate from early November to late February. They emerge in early March, and start courting and mating from April. Oogenesis started in late April, raises from Late May to late June and ended in late Julay.

In this species both of ovaries are active. In the early stage of oogenesis the mean diameter of oocytes were 2.95 and 2.82 mm in right and left ovaries, respectively. All of oocytes grow but only some of them reach the maximum size and become shelled eggs. The volume of oocytes increases between June and July. Some oocytes enter vitellogenesis and grow. During vitellogenesis, oocytes enter the oviducts and fertilization occurs in spring. At the end of vitellogenesis, the mean diameter of them are 13.31 and 12.54 mm in right and in left ovaries, respectively. Oviposition occurs in June and July and juveniles hatch in mid August to September. Snakes return to their hibernation sites in mid October to early November. In this study the mean length of females was 102.145 cm. As spring approaches, the males emerge first and spend much of the day basking in an effort to raise their body temperature and thereby their metabolism. This may be a tactic to maximize sperm production as the males mate with the females as soon as they emerge up to 2 weeks later in April or earlier if environmental temperatures are favorable. The mean weights of the right and left ovary were 5.01 and 3.56 g, respectively. The number of oocytes depends on body size and varies from 7-39 in right ovaries and 3-36 in left ovaries. The mean oocyte numbers in the right and left ovaries were 18.60 and 14.87, respectively. Ovaries can produce different numbers of eggs in each year.

There are significant differences in body weight and length, ovary weight and diameter, oocyte number, weight and diameter and GI in different months. There are significant differences in follicle diameter and number and follicular layer diameter in left and right ovaries. Table 1 shows the descriptive of characters.

In Iran, on the basis of size and ecological conditions the number of oviductal eggs varied from 1 to 12 (Figure 4). The Maximum numbers of oviductal in both oviducts were 20 which observed in a female with W = 339.42 g, SVL = 82.5 and LCD = 19.15 cm in late June and the minimum number of oviductal eggs was 4, observed in a female with W= 83.82 g, SVL = 48.50 and LCD = 14.10 cm in mid July. The mean length and width of right oviductal eggs are 35.08 and 18.05 mm and the mean length and width of left oviductal eggs are 32.22 and 19.05 mm, respectively. The mean weight of eggs was 6.03 g.

**Table 1. Descriptive statistics for the examined characters in *N. tessellata tessellata*.**

| Characters | N | Range | Minimum | Maximum | Mean Statistic | Std. Error | Std. Deviation | Variance |
|---|---|---|---|---|---|---|---|---|
| W (g) | 63 | 279.00 | 60.42 | 339.42 | 175.7017 | 9.63062 | 76.44064 | 5843.172 |
| SVL (cm) | 63 | 255.75 | 46.50 | 111.25 | 102.1454 | 10.31573 | 81.87856 | 6704.099 |
| LCD (cm) | 63 | 8.00 | 11.50 | 19.50 | 15.3276 | 0.25260 | 2.00494 | 4.020 |
| ROD (mm) | 63 | 224.27 | 15.58 | 239.85 | 73.5921 | 5.30331 | 42.09370 | 1771.880 |
| LOD (mm) | 63 | 121.11 | 10.11 | 131.22 | 55.7749 | 3.84629 | 30.52901 | 932.020 |
| ROW (g) | 63 | 49.74 | 0.08 | 49.82 | 5.0150 | 1.22406 | 9.71571 | 94.395 |
| LOW (g) | 63 | 33.90 | 0.04 | 33.94 | 3.5696 | 0.90123 | 7.15329 | 51.170 |
| RFN | 63 | 32.00 | 7.00 | 39.00 | 18.6032 | 1.03338 | 8.20216 | 67.275 |
| LFN | 63 | 33.00 | 3.00 | 36.00 | 14.8730 | 0.85703 | 6.80249 | 46.274 |
| MaxRF (mm) | 63 | 39.15 | 4.20 | 43.35 | 13.3107 | 1.36916 | 10.86735 | 118.099 |
| MaxLF (mm) | 63 | 33.45 | 3.89 | 37.34 | 12.5412 | 1.22288 | 9.70632 | 94.213 |
| MinRF (mm) | 63 | 5.19 | 1.18 | 6.37 | 2.9513 | 0.10561 | 0.83826 | 0.703 |
| MinLF (mm) | 63 | 4.62 | 1.31 | 5.93 | 2.8263 | 0.10590 | 0.84054 | 0.707 |
| MRF (mm) | 63 | 19.93 | 2.81 | 22.74 | 6.8813 | 0.51101 | 4.05604 | 16.451 |
| MLF (mm) | 63 | 16.85 | 2.67 | 19.52 | 6.2743 | 0.43814 | 3.47765 | 12.094 |
| GI | 63 | 42.70 | 0.10 | 42.80 | 4.3730 | 0.99422 | 7.89138 | 62.274 |
| MDRFL (μ) | 63 | 124.41 | 28.00 | 152.41 | 77.4541 | 2.78810 | 22.12983 | 489.729 |
| MDLFL(μ) | 63 | 93.00 | 28.00 | 121.00 | 75.1592 | 2.29566 | 18.07608 | 326.745 |
| MRTA (μ) | 63 | 18.50 | 13.00 | 31.50 | 19.1340 | 0.48794 | 3.87287 | 14.999 |
| MLTA (μ) | 63 | 15.50 | 13.00 | 28.50 | 20.0808 | 0.52681 | 4.18142 | 17.484 |

**Figure 4: Oviductal eggs of *N. tessellata tessellata* in late June**

**Figure 5: Juvenile of *N. tessellata tessellata* during hatching in 5 August**

**Figure 6: Juvenile of *N. tessellata tessellata* after hatching in 5 August**

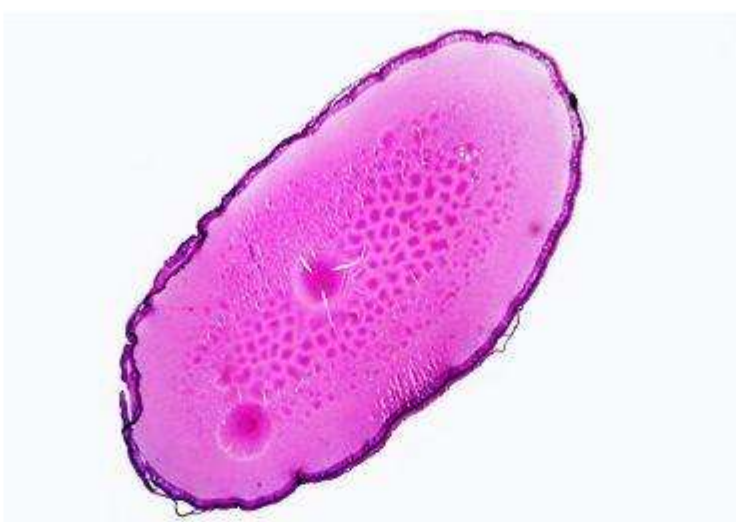

**Figure 7: Cross section of oocyte of *N. tessellata tessellata* in August (*Photos by A. Soofizadeh)***

Females often chose compost and manure heaps as the warmth of these substrates acted as a natural incubator. The eggs are laid from June to July in a moist, warm spot, usually under or in rotting wood piles and other such places. Sites with rotting vegetation, such as compost heaps are preferred locations. Leathery-skinned mattwhite eggs were hatched after about 10 weeks (Figure 5). The smallest juvenile (SVL = 18.62 and LCD = 4.65 cm) was observed in early August (Figure 6). The cross section of oocyte was prepared (Figure 7).

***Discussion***

The maximum body length of females in this study was 111 cm, whereas in other studies, it has been recorded 103 cm (Farzanpey, 1990) and 124 cm (Latifi, 1991). The snakes are about 50 cm long when they reach sexual maturity because the specimen with SVL = 48.50 cm had oviductal eggs. Also the numbers of eggs in this study was 4 - 12, but in other studies has been recorded 5 – 12 (Latifi, 1991; Farzanpey, 1990), 35 (Areste and Cebrian, 2003), 10 – 25 (Herczeg *et al.*, 2005), 10 – 30 (Vlcek *et al.*, 2010; Mebert, 2011). These differences may be because of different geographical conditions among populations of this species.

*N. tessellata* shows some differences with other watersnakes such as *N. natrix* and *N. maura*. The maximum body length of females of grass snake, *N. natrix*, has been recorded 122 cm (Latifi, 1991), 100 cm (Farzanpey, 1990), 106 cm (Shiravi *et al.*, 2012) and in *N. maura* has been recorded 75 cm (Mattison, 1999). The number of eggs of *N. natrix* has been recorded 6 – 12 (Latifi, 1991), 6 – 13 (Farzanpey, 1990) and 4 – 13 (Shiravi *et al.*, 2012) in Iran and 10 – 40 eggs in England (Areste and Cebrian, 2003). The females of *N. natrix* mate every other year in England and they lay eggs and incubate them in their burrows for around 10 – 12 weeks until they hatch in late summer in August or September (Gregory and Isaac, 2004). The numbers of eggs in *N. natrix*, have been recorded 11 – 53 (Areste and Cebrian, 2003), 30 – 45 (Madsen, 1987), 11 – 48 (Mertens, 1995) and has been recorded 24 in *N. maura* (Santos and Llorente, 2001). The oogenesis of *N. tessellata* is similar with *N. natrix* and starts at April but oviductal eggs in *N. tessellata* was observed from mid May and in *N. natrix* have been observed from early June (Shiravi *et al.*, 2012).

The oviposition time of *N. tessellata* in this study was recorded from late June to early August and in other studies have been recorded from June to August (Areste, 2003), July to August (Vlcek, 2010; Mebert, 2011) and May to June (Herczeg *et al.*, 2005). In other water snakes such as *N. natrix* the oviposition time have been recorded from July toAugust (Shiravi *et al.*, 2012) and from June to August (Areste, 2003), and in *N. maura* has been recorded from July to September (Santos and Llorente, 2001).

Females are larger in spring due to their eggs. The eggs incubate for around 10 weeks before hatching. To survive and hatch the eggs require a temperature of at least 22°C, but preferably 30°C, with high humidity. The young are about 18 – 19 cm long when they hatch and are immediately independent; however, they are eaten by a wide range of animals. Other research on reproductive cycle of viperine snakes, *Natrix maura*, showed that they emerged from hibernation in March and mating occurred from April to June, using sperm stored by males over winter (Santos *et al.*, 2000). Vitellogenesis was prenuptial as follicular growth occurred after emergence from hibernation. Gravid females were found in July and August (Santos *et al.*, 2000). The interpopulational differences may be related to variation in prey availability and variation in the lipid content of fat bodies supports this hypothesis (Santos *et al.*, 2000). In April, both males and females showed few lipid reserves, and vitellogenesis was delayed to the extent that females were gravid in August (Santos *et al.*, 2000). In contrast to other snake species, variation in food availability did not affect the proportion of reproductive females or clutch size (Santos *et al.*, 2000). Nonetheless, even after adjusting for such seasonal variation, gravid females contained food less frequently than nongravid females (Gregory and Isaac, 2004). Because ectothermy facilitates long-term energy storage and often involves low feeding rates, traditional views suggest that many ectotherms rely heavily on stored reserves for egg production (Warner *et al.*, 2008). Females utilize both endogenous energy stores and recently acquired food to fuel reproduction; this pattern did not shift seasonally from first to second clutches produced. Importantly, however, egg lipid was derived primarily from stored reserves, whereas egg protein was derived about equally from both recently acquired energy and stored reserves (Warner *et al.,* 2008). Many reptiles are typical capital breeders that fuel reproduction by the use of lipids stored in fat bodies (Santos *et al.,* 2007). The origin of egg protein was examined and results showed that protein in liver peaked during vitellogenesis according to the role of the liver in the synthesis of vitellogenin (Santos *et al.,* 2007). Partial correlations showed the path of protein from the prey (digestive-content) to the liver, and finally to the ovaries, as well as an inverse relation between carcass protein and ovarian mass. Carcass muscle is the only body part that may act as a potential reserve for endogenous protein, although they did not find significant variation in carcass protein during female reproduction. As females with large follicles did not stop foraging activity, probably egg protein was derived from the diet as partial correlations indicated (Santos *et al.*, 2007). *N. maura* is a capital breeder for lipids and tend to be income breeder for protein. This conclusion contrasts with that observed in capital breeders for which egg protein was derived from muscle (Santos *et al.*, 2007). Flexibility in the origin of egg protein could affect the body condition in postreproductive females (Santos *et al.,* 2007). Studying the reproductive cycle in different parts of a snake's range allows us to see the extent of geographic variation in reproduction within a species or family. Of the climatic factors likely to influence reproduction, only temperatures low enough to require prolonged hibernation play any part in the determination of the reproductive cycle. In regions where there is no prolonged hibernation, male sexual activity may occur at any time of the year, depending on the reproductive cycle of the females. However, it is always dependent upon the climate (Saint Girons, 1982). Storage of sperm in the vas deferens of males may be indispensable in the reproductive cycles of aestival type, but prolonged retention of sperm in the oviducts, while permitting fertilization in the absence of males, plays only a very minor role in the harmonization of the reproductive cycles of both sexes in the snakes (Saint Girons, 1982). Our results show that the reproductive cycle of *N. tessellata* similar to *N. natrix* in northern Iran is alternate and occurred during the well defined period in which oocytes were not found in the ovaries all year.

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