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Variation of clutch characteristics in population of Eastern Hermann's tortoises (*Testudo hermanni boettgeri* Gmelin 1789)

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***Abstract*:**

Stojadinović, D., Vidojević, D., Crnobrnja-Isailović, J.: ***Variation of clutch characteristics in population of Eastern Hermann's tortoises (Testudo hermanni boettgeri Gmelin 1789). Biologica Nyssana, 8 (1), September 2017: 123-127.***

We present here information on some fecundity parameters in local population of Eastern Hermann's tortoise situated in the area of Kunovica, at the outskirts of the city of Niš. The data were collected in the field and in 97% were based on reconstruction of number and dimensions of eggs in destroyed nests what influenced on quality of information. Only two of 78 nests recorded from 2010 to 2014 were found intact. Other 76 clutches were found destroyed and therefore only some fecundity parameters were measurable, while the others were reconstructed by using formulas proposed in the relevant literature. On the basis of collected data we estimated average clutch size of Hermann's tortoises in Kunovica as four eggs what was lower than in populations from Greece. However, the most of calculated egg dimensions (e.g. maximal width, volume and mass) were higher than in populations from Greece and France.

Key words: fecundity, clutch size, egg size, *Testudo hermanni boettgeri*

***Apstrakt*:**

Stojadinović, D., Vidojević, D., Crnobrnja-Isailović, J.: ***Varijacije karakteristika legla u populaciji istočne podvrste šumske kornjače (Testudo hermanni boettgeri Gmelin 1789). Biologica Nyssana, 8 (1), Septembar 2017: 123-127.***

U ovom radu predstavljene su informacije o nekim parametrima fekunditeta lokalne populacije istočne podvrste šumske kornjače sa područja Kunovice (okolina grada Niša). Svi podaci su prikupljeni na terenu i u 97% slučajeva izvršena je rekonstrukcija broja i dimenzija jaja, što utiče na kvalitet informacija. Samo dva od 78 pronađenih gnezda u periodu od 2010. do 2014. su bila sa neoštećenim jajima. Ostala gnezda su bila uništena, tako da su samo neki parametri mogli da budu izmereni, dok su ostali rekonstruisani pomoću formula datih u odgovarajućoj literaturi. Na osnovu prikupljenih podataka, procenili smo da je prosečna veličina legla kod šumske kornjače iz Kunovice 4 jaja, što je manje nego kod populacija iz Grčke. Ipak, većina karakteristika jaja ove lokalne populacije (npr. maksimalna širina, zapremina, masa) je bila veća nego kod populacija iz Grčke i Francuske.

Ključne reči: fekunditet, veličina legla, veličina jaja, *Testudo hermanni boettgeri*

Introduction

Traditional life-history approach (Gadgil & Bossert, 1970) proposes that reproductive investment increases with age and reaches the peak in the act of terminal investment, where reproduction stops afterwards (Williams, 1966; Hirschfield & Tinkle 1975; Clutton-Brock, 1984). Other theory proposes that reproductive investment depends on number of characters subjected to various rates of change, what depends on age of the individual (McNamara & Houston, 1996). Reptiles in general have greater and more variable fecundity in comparison to endothermous organisms (Shine, 2005). In general, female's fecundity could vary on different levels: within individual female's life, among females within the same population, among females from conspecific populations or among females of different species. In many reptiles, larger females produce bigger clutches (Fitch, 1970). Reproduction is usually delayed in females until they accumulate enough energy to produce clutch as large as their abdominal cavity is (Vitt & Congdon, 1978; Vitt & Price, 1982). Some observations confirmed that decrease of average female body size and average clutch size, caused by environmental change, may decrease survival and growth rates (Shine, 2005).

Egg laying happens from middle May to end of June (Cruce & Răducan, 1976; Swingland & Stubbs, 1985; Fertard, 1992; Bertoleroet al., 2007b) and females repeatedly use the same nesting place (Swingland & Stubbs, 1985). Incubation period varies from 90 to 124 days (Cruce & Răducan, 1976; Cheylan, 1981; Nougarède, 1998). Eggs are of white color, hard shelled and almost ellyptical in shape. Incubation temperature varies from 23°C, to 34°C (Eendebak, 1995). If incubation temperature keeps constantly at 25°C or 33-34°C, mortality of the embrios increases to approximately 50%. On the temperatures lower than 23°C or higher than 34°C mortality reaches 100% (Eendebak, 1995).

In year 2010 we established regular monitoring of one local population of Eastern Hermann's tortoise in the area of village Kunovica in vicinity of town of Niš in South-Eastern Serbia. Our main goal was to collect data on life history parameters and to record their variation in order to enable population viability analysis in the future. This study presents the most possible projection of female fecundity in population of Hermann's tortoises in Kunovica based on data collected during first five years of monitoring. We assumed that average clutch size and egg dimensions could be in range of values reported for other analysed populations of *Testudo hermanni boettgeri*.

Material and methods

Field procedures

Field work was conducted twice per year from 2010 to 2014, always in May and June and seven consecutive days each. Detailed description of the studied area could be find in Stojadinović et al. (2013). During the day researchers were collecting records on different aspects of biology of the species, eggs laying and nests. Clutch size was estimated by reconstruction of located destroyed nests only where counting of eggs from shells was possible. Direct counting of eggs was possible when females were encountered while laying eggs. In these two cases the eggs from intact nest were carefully taken from the hole, measured for mass and maximal lenght & width by Pesola field balance with accuracy of 0.2 g and by digital calliper with precision of 0.01 mm, respectively. The eggs were carefully deposited back in the nest afterwards and covered with soil. We considered egg dimensions as reliable only when length and/or maximal width of egg shells were available to measure and they were included in the analysis.

Species description

Testudo hermanni (Gmelin 1789) is one of three European tortoise species. Two subspecies of Hermann's tortoise are currently recognized: The western subspecies -*T. hermanni hermanni*- inhabits parts of Spain, France and Italy while eastern subspecies -*T. hermanni boettgeri*- is distributed in Croatia, Bosnia and Herzegovina, Montenegro, Albania, Serbia, FYRM, Romania, Bulgaria, Greece and Turkey (Fritz et al. 2006).

Statistical procedures

Egg volume (EV, mm³) was estimated by applying the equation $\mathbf{EV=(\pi*L*w^2)/6000}$ (Longepierre et al., 2003), where π =3.14, L= longer egg axis or egg

Table 1. Overview of fecundity parameters recorded in Hermann's tortoise populations from different parts of species range (France, Greece) and from Serbia. n – number of analyzed nests, N_{av} – average clutch size, L_{av} – average egg lenght/clutch, W_{av}- average maximal egg width/clutch, $(L/W)_{av}$ – average value of egg shape/clutch, EV_{av} - average value of egg volume/clutch, EM_{av} – average value of egg mass/clutch. s.d.-standard devitiation.

	France 1 (Longipierre, 2003)	France 2 (Longipierre, 2003)	Greece 1 (Hailey, 1988)	Greece 2 (Hailey, 1988)	Greece 3 (Hailey, 1988)	Serbia (our data, destroyed clutches)	Serbia (our data, intact clutches)
n						76	2
N_{av} ±s.d.	3 ± 1	3 ± 1	5 ± 1	5 ± 1	5 ± 1	4 ± 1	4.5±0.71
L_{av}±s.d.	35.6	-	37.1± 2.2	37.5 ± 2.2	38.8± 2.7	37.1± 2.3	37.4±0.7
w_{av}±s.d.	27.8	-	27.0±1.5	28.9 ± 0.9	28.0± 1.7	29.8± 2.9	30.3±0.4
$(L/w)_{av}$±s.d.	1.28	-	1.37± 1.5	1.3 ± 1.5	1.38± 1.5	1.25± 0.1	1.2±0.0
EM_{av}	16.5	15.8	16.24	18.57	18.09	19.6	20.0
EVav	14.4	-	14.15	16.39	15.92	17.5	17.9

lenght, and w = shorter egg axis or egg width (Coleman, 1991). Egg mass (EM, g) was estimated by applying the equation **$EM=0.435*L+0.0203*w^2-14.7$** (Hailey & Lombourdis, 1990), where L = egg lenght, and w = egg width. Average egg size, shape (L/w), mass and volume were estimated independently for every nest (Longepierre et al., 2003).

All statistical analyses were done by software package Statistica 7.0 (StatSoft Inc.).

Results and discussion

Only two of 78 Hermann's tortoise clutches recorded during four years of monitoring field site in Kunovica were found intact and they contained four and five eggs. Females that deposited these clutches were measured for streight carapace length (SCL), mid-body carapace width (MCW), maximal carapace width (MacCW) and weight (W). For female that deposited four eggs measures were: SCL-193.58 mm, MCW-141.38 mm, MaxCW-143.42 mm, W-1347 g; for female that deposited five eggs measures were: SCL- 184.15 mm, MCW- 145.93 mm, MaxCW-146.13 mm, W-1390 g. Average egg lenght in intact clutch was 37.40 mm ± 0.68 mm, average maximal width was 30.25 mm ± 0.39 mm, and average egg mass was 20.0 g ± 0.68 g.

The reconstruction of clutch and egg size enabled us to appoximate average clutch size (4 eggs ± 1 egg) in this particular population of Eastern Hermann's tortoise. Clutch size varied from 1 to 7 eggs. Overall number of nests recorded over years was 13 in 2010 (6 in last week of May and 7 in third week of July), 6 in 2011 (4 nests in May and 2in July), 4 in 2012 (4 nests in May), 38 in 2013 (35 in May and 3 in July) and 17 in 2014 (4 nests in May and 13 in July).

Using Hailey and Lombourdis (1990) formula for estimation of egg mass on the basis of egg dimensions we got hypothetic average egg mass value of 20.8 g ± 4.8 g. Observed average egg mass recorded in the field (based on measured intact eggs) was 20.0 g ± 0.68g. Additionally, we have also calculated mean values of fecundity parameters for destroyed clutches, and presented their averages in Table 1. Average egg length per clutch was 37.1 mm, varying from 33.4 mm to 41.3 mm. Average value of maximum egg width per clutch was 29.8 mm, varying from 24.0 mm to 35.4 mm. Average approximate egg mass per clutch was 19.6 g, varying from 11.8 g to 26.6 g. Average egg volume per clutch was 17.5 mm^3, varying from 10.3 mm^3 to 24.5 mm^3.

Clutch size in *Testudo hermanni* varied from one to seven eggs in western (nominative) subspecies (Esteban, 1987; Fertard, 1992; Nougarède, 1998; Longepierre et al.,2003; Bertolero et al., 2007c) and from one to nine eggs in eastern (*boettgeri*) subspecies (Swingland & Stubbs, 1985; Hailey & Loumbourdis, 1988, 1990) (**Tab. 1**). Average clutch size varied from 3.3 in western (Esteban, 1987; Fertard, 1992; Nougarède, 1998; Longepierre et al., 2003; Bertolero et al., 2007c) to 4.3 eggs in eastern subspecies (Swingland & Stubbs, 1985; Hailey & Loumbourdis, 1988, 1990).

Average egg size and weight were 27.2× 34.3 mm and 15.1 g, respectively, in *Testudo hermanni hermanni* (Cheylan, 1981; Esteban, 1987; Fertard, 1992; Longe-pierre et al., 2003; Bertolero et al., 2007a,c) and 27,9×37,4 mm and 17.1 g, respectively, in *Testudo hermanni boettgeri*

(Cruce & Răducan, 1976; Hailey & Loumbourdis, 1988, 1990;).

In general, reproductive traits in female Hermann's tortoises were related to body size and the biggest females have the greatest contribution to overall egg production per reproductive season (Cruce & Răducan, 1976; Hailey & Loumbourdis, 1988; Fertard, 1992; Longepierre et al.,2003; Bertolero et al., 2007c). It was not possible to test this relation in our study as it was almost completely based on data obtained from inspection of broken nests. In fact, only two of 78 recorded tortoise nests were found intact (females were spotted while laying the eggs in May 2010). Nonparametric Kolmogorov-Smirnov two-sample test has shown that there was no significant difference between body size and mass of these two females nor between egg parameters in these two clutches.

Except 2014, in other years of study the most of the nests was detected in May. It could be explained by the fact that high environmental temperatures starting from July could influence on egg laying time in reptiles with multiple clutches e.g. temperature increase results in earlier nesting time in the summer.

Studies conducted in France and Greece suggested that the first egg laying occursin the mid-April, while the last was detected in July. Particular flexibility was detected in beginning and the end of the nesting period what could be related to variation in environmental conditions (Cruce & Raducan, 1976). Radiological surveys in those two countries confirmed that the highest number of calcified eggs in oviducts was detected during May and June (Bertolero et al., 2007b).

Hailey and Loumbourdis (1988) were studying reproduction of Hermann's tortoise on three different localities in Greece (Alyki, Litochoron and Deskati). Average clutch size was 5 eggs (Table1). The lowest values of analysed fecundity parameters were recorded on the first locality – dry sandy ground. The other two localities were on fertile soil and similar regading analysed fecundity parameters. Localities in France (Plaine des Mauresand Massif des Maures) were under the influence of Mediterranean climate (Longepierre et al., 2003) what could explain their lower values of fecundity parameters in comparison to Greece and Serbia (**Tab. 1**).

The largest Hermann's tortoise eggs were recorded in Serbia (in comparison with data from France and Greece, see **Tab. 1**) which should be taken with caution since most of the data were obtained from broken egg shells. Egg size could be correlated to body size, but it is not the rule: in some populations larger female tortoises have wider eggs because egg size is constrained by width of the pelvic channel (Congdon & Tinkle, 1982). However, in some populations smaller females lay more narrow eggs but similar in weight to those of larger females (Halilev & Loumbourdis, 1988).

Conclusion

Reconstruction of number and dimensions of eggs in destroyed nests of Hermann's tortoise from Kunovica showed values similar to those obtained from measurement of two intact nests Estimated average clutch size of female Hermann's tortoises in Kunovica was lower than in populations from Greece, but higher than in populations from France. Maximal width, volume and mass of eggs were higher than in population in Greece and France.

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