

Morphology and reproduction of the Snake-eyed Skink (*Ablepharus kitaibelii* Bibron & Bory De Saint-Vincent, 1833) in the western most parts of its range

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Received: 28. January 2017 / Accepted: 14. September 2017 / Available online: 23. September 2017 / Printed: December 2018

Abstract. The most western populations of the Snake-eyed Skink, discovered recently on Papuk Mountain and Ilok area in Croatia, has never been studied before. Here we examined the morphology and age structure, reproduction, and prevalence of injuries in both populations. We examined 191 individuals: 163 adults and 28 juveniles. Morphological analysis was based on 140 adult individuals (129 from Papuk, 11 from Ilok) and 34 juveniles (21 caught in the wild and 13 hatched in captivity). Our results showed that although there is no clear sexual dimorphism, adult animals exhibit slight differences between sexes. In 34.1 % of the adult individuals in Papuk and in 35 % of juveniles, a tail has been regenerated, while in Ilok in 27.3 % of the adults. In 2010-2012 nine gravid females collected from Papuk Mountain deposited a total of 25 eggs in the lab. The number of eggs in each clutch was recorded, and each egg was weighed and measured regularly until hatching. Clutch size spanned between 2-4 eggs with an average of 2.78 eggs. Nine eggs did not develop. Monitoring of egg sizes showed that average length, width, mass, surface and volume increase linearly during the incubation time, but the growth was allometric. The larger size of a female does not result in an increased number of eggs, but in an increased individual egg size.

Key words: Reptilia: Squamata, Sauria: Scincidae, sexual dimorphism, Ilok, Papuk.

Introduction

The Snake-eyed Skink, *Ablepharus kitaibelii* (Bibron and Bory de Saint-Vincent, 1833) is the only representative of family Scincidae in Central Europe (Herczeg et al. 2004). It is distributed from Slovakia in the north, through the Balkan Peninsula to the south, reaching central-Turkey to the east (Gruber 1981, Schmidtler 1997). The most western population of the Snake-eyed Skink was discovered just recently on Papuk Mountain in Croatia, and the existence of population in Ilok area on the east of Croatia was also reconfirmed (Szövényi & Jelić 2011).

The Snake-eyed Skink is one of the smallest lizards in Europe with a snout-vent length (SVL) from 20 to 55 mm in adult individuals, weighing from 0.15 to 1.5 g (Herczeg et al. 2007). In the Carpathian Basin the Snake-eyed Skink can be found on various habitat types: from steppe and shrubs to sub-Mediterranean deciduous forest (Ljubisavljević et al. 2002), including different bedrocks (e.g. limestone, sandstone, basalt, loess, calcareous sand; Harnos & Herczeg 2003, Herczeg et al. 2004). Mating of the Snake-eyed Skink takes place in April and May (Fejerváry 1912, Gruber 1981), egg deposition starts in June and continues until mid-August, and hatching occurs from August throughout September.

In our research, we aimed at obtaining data on several aspects of the ecology of Snake-eyed Skink. Firstly, we studied the morphology and the age structure of individuals of the Snake-eyed Skink from two populations in Croatia (Papuk Mountain and Ilok). Since both populations are isolated from each other, and from the main population, we can expect that some differences between them could be observed. Within that part of our study, we also examined the prevalence of injuries in both populations. Secondly, we wanted to gain better insight into the species reproduction. Although some general data on its reproduction are known,

it is possible that our populations differ from other populations due to their isolation and small population size, but also due to different habitat conditions. These data are of great importance not only because general knowledge on this species is relatively poor, but also in order to apply the right conservation measures.

Materials and methods

Site description

Data on the individuals of the Snake-eyed Skink were collected from both known localities of this species in Croatia, from Papuk Mountain and from Ilok. The site of Papuk Mt. (N 45.470000°; E 17.630000°) includes the area in the southern part, near the botanical reserve, which is covered by rear thermophilous forest community of *Quercus pubescens* Willd, 1796 with *Fraxinus ornus* Linnaeus, 1753 and *Juniperus communis* Linnaeus, 1753. The localities in Ilok are very fragmented, and the majority of individuals were recorded on the hills of the main City Park (N 45.220000°; E 19.370000°) in a habitat that is somewhat similar to that on Papuk (thermophilous plants), but also includes areas covered by invasive species such as *Ailanthus altissima* (Miller) Swingle, 1916 and *Amorpha fruticosa* Linnaeus, 1753. These trees replaced the original forest of *Quercus pubescens* (known from historic descriptions), but still provide favourable habitat. It seems that in both sites (Papuk and Ilok) size of the favourable habitat is just 3-5 ha and populations are limited to just several thousands of individuals each (best scientific judgement).

Morphology

Fieldwork was carried out between 2009 and 2012 both in Papuk Mountain and in Ilok, for a few consecutive days, from June to October. All the individuals were captured by hand. Each individual was photographed from all sides and visually inspected for injuries and tail morphology (presence/absence, regrown). It was assigned into an age group (juvenile/adult) based on its snout-vent length (SVL in juveniles up to 30 mm), and body and tail colouration (a red-orange tail colouration is indicative of juveniles; Fig. 1). We measured body mass (BM) and following morphological data of each individual: total length (TL), SVL, tail length (TLL), head length (HL), snout to

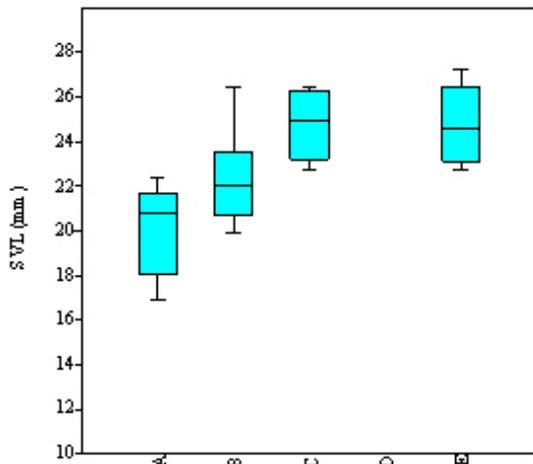


Figure 1. Red coloration on the tail of juvenile individuals shown in categories based on SVL (A and B, SVL from 16.87 to 26.45 mm); A) newly hatched individuals with intensively red tails, max age of 24 hours (n=6); B) juvenile individuals from the wild with intensively red tails (n=8); C) juvenile individuals with reduced red coloration ventrally or/and dorsally (SVL from 22.77 up to 26.41 mm); D) juvenile individuals without red coloration (n=1; SVL = 26.38 mm); E) juvenile individuals whose tail colouration could not be determined (e.g. tail was missing) (n=5).

forelimb length (SFL - measured from snout tip to beginning of forelimb base), forelimb to hindlimb length (FHL - measured from inner margin of base of forelimb to inner base of hindlimb), maximum head height (HH) and maximum head width (HW). The measurements were taken with a vernier hand calliper with precision of 0.01 mm. Body mass was recorded with a digital scale with the precision of 0.01 g. Nine individuals from Papuk were euthanized using Chloroform; their sex was confirmed by gonad inspection.

Reproduction

In July of 2010-2012 a total of 21 gravid females were collected from Papuk Mt. to obtain data on the species reproduction. The study was carried out in the facilities of Zagreb Zoo. Gravid females were kept in separate terraria, under conditions that imitate the natural habitat, with provided shelters and a bottom covered with 2 cm of soil mixed with some vermiculite. Females were fed daily *ad libitum* with crickets, fruit flies and occasionally mealworm larvae. Temperature, humidity and drinking water were monitored daily, and humidity was maintained by spraying manually (~80%). Females were weighted daily. After the egg deposition, seven females were sacrificed and their gonads were examined in detail to obtain data on follicle condition and number of eggs present.

Within 24 hour after oviposition, the number of eggs in each clutch was recorded, and each egg was weighed, and its maximal length and maximal width were measured. These measurements were then taken every few days until the hatching of juveniles. The eggs were marked and transferred to incubation chambers without changing their relative position, and each clutch was kept in a separate container, filled with vermiculite (150 g water per 100 g vermiculite). The eggs were incubated at 28°C, under 80 % air humidity. After two weeks of incubation, each egg was covered with a transparent plastic cup in order to allocate each juvenile to its egg. Just after the hatching, the same morphometric measurements that were taken on adults in the field were also taken on juveniles.

Data analysis

For the morphometric data obtained we calculated basic descriptive statistics: minimum (MIN), maximum (MAX), average (AVR) and standard error (SE). Data for TL and TLL for those individuals that had amputated or partial tail were excluded from the analysis. We

also calculated the following ratios: HL/SVL, SFL/SVL, FHL/SVL, HH/SVL, and HW/SVL, which were then submitted to discriminant analysis and PCA. Statistical analysis were carried out using software PAST v. 2.06 and Microsoft Excel 2007.

Relative clutch mass (RCM) was calculated as the ratio of the total clutch mass and the mass of the gravid female (Aleksić & Ljubisavljević 2001, Vrcibradic & Rocha 2002).

We also estimated the volume (V) and the surface (S) of the eggs. The calculation was based on the assumption that the eggs have a shape of elongated spheroid, and the following formulas were applied (following Kratochvíl & Frynta 2006):

$$V = \frac{4}{3} \pi a b^2 \quad \text{i.e.} \quad V = \frac{4}{3} \pi \left(\frac{1}{2} L\right) \left(\frac{1}{2} W\right)^2$$

$$S = 2\pi b \left(b + \frac{a \cdot \arcsin[e]}{e}\right), \quad e = \frac{(a^2 - b^2)^{1/2}}{a}$$

where: b - half of maximal width (W) of the egg (the shortest diameter of ellipsoid)

a - half of maximal length (L) of the egg (the longest diameter of ellipsoid)

The comparison of female size and the size of its clutch was analysed using linear correlation models.

Results

Population age structure

We recorded 191 individuals of the Snake-eyed Skink. 163 were assigned as adults (152 from Papuk, 11 from Ilok) and 28 as juveniles (27 from Papuk, one from Ilok). Although collection was haphazardous, and occurred over the whole research area, it is possible that in a limited number of cases the same individuals were captured more than once, but this would not have any influence on morphological data quality. The first juveniles were recorded on 2 August and they appeared up to October in Papuk, while the only juvenile individual from Ilok was found in September. In autumn, the number of adult and juvenile individual was approximately equal, while in spring the number of adult individuals was much higher.

Morphological characteristics

Morphological analysis was based on 140 adult individuals (129 from Papuk, 11 from Ilok; Table 1). Morphological analysis of juvenile individuals was based on 34 individuals, 21 caught in the wild (20 from Papuk, one from Ilok) and 13 hatched in captivity. Morphometric measurements of juvenile individuals are shown separately for the individuals caught in the wild since their age was not known (Table 2), and for the individuals hatched in the captivity (Table 3).

Adult animals exhibit slight differences between males and females but border values are overlapping. The discriminant analysis indicated 50.71 % of correct gender identification in our sample (Table 4a). The most significant discrimination was shown by L_{SFL}/L_{SVL} and L_{FHL}/L_{SVL} (lower in females), followed by L_{FHL}/L_{SVL} (lower in males; Table 4b). Males have a proportionally larger head (HL/SVL = 16.9 % and SFL/SVL = 29.9 %) compared to the body (FHL/SVL = 63.8 %), while in females it is the opposite (HL/SVL = 13.9 % and SFL/SVL = 24.6 %; FHL/SVL = 65.8 %). PCA analysis (Table 5) was performed with discriminated groups resulting from the discriminant analysis (Table 4a). Figure 2 shows

Table 1. Morphometric measurements of adult individuals from Papuk and Ilok. Number of individuals (n), average (MEAN), standard error (SE), minimum (MIN), maximum (MAX). See materials and methods for other abbreviations.

| | Papuk | | | | Ilok | | | | Mann -Whitney U | |
|------------|-------|---------------|-------|--------|------|----------------|-------|--------|-----------------|-------|
| | n | Mean (1SE) | Min | Max | n | Mean (1SE) | Min | Max | U | p |
| TL (mm) | 79 | 98.48 (1.349) | 61.80 | 123.08 | 8 | 110.61 (4.512) | 90.10 | 126.47 | 162 | 0.021 |
| SVL (mm) | 129 | 43.99 (0.417) | 33.36 | 62.64 | 11 | 46.32 (1.400) | 39.4 | 52.38 | 505 | 0.114 |
| TLL (mm) | 79 | 54.79 (1.274) | 17.38 | 70.47 | 8 | 76.73 (76.73) | 49.21 | 78.12 | 185.5 | 0.057 |
| HL (mm) | 129 | 6.74 (0.034) | 5.73 | 7.64 | 10 | 6.38 (0.104) | 5.62 | 6.83 | 305 | 0.006 |
| SFL (mm) | 129 | 11.89 (0.073) | 10.18 | 14.10 | 11 | 11.82 (0.259) | 10.01 | 13.00 | 672 | 0.771 |
| FHL (mm) | 129 | 28.74 (0.354) | 19.30 | 38.76 | 11 | 31.11 (1.309) | 23.76 | 36.69 | 487 | 0.085 |
| HH (mm) | 115 | 2.94 (0.022) | 2.18 | 3.73 | 11 | 2.87 (0.053) | 2.59 | 3.09 | 533 | 0.390 |
| HW (mm) | 115 | 4.23 (0.035) | 2.04 | 5.07 | 11 | 4.04 (0.088) | 3.65 | 4.55 | 414 | 0.060 |
| Weight (g) | 92 | 1.19 (0.033) | 0.54 | 2.00 | 5 | 1.37 (0.170) | 0.98 | 1.98 | 169 | 0.329 |
| HL/SVL | 129 | 0.155 (0.001) | 0.107 | 0.198 | 10 | 0.140 (0.005) | 0.118 | 0.163 | 331 | 0.008 |
| SFL/SVL | 129 | 0.273 (0.002) | 0.194 | 0.336 | 11 | 0.257 (0.009) | 0.200 | 0.307 | 499 | 0.103 |
| FHL/SVL | 129 | 0.652 (0.004) | 0.523 | 0.825 | 11 | 0.670 (0.011) | 0.603 | 0.719 | 507 | 0.120 |
| HH/SVL | 115 | 0.068 (0.001) | 0.048 | 0.094 | 11 | 0.062 (0.002) | 0.053 | 0.072 | 368 | 0.022 |
| HW/SVL | 115 | 0.098 (0.001) | 0.046 | 0.129 | 11 | 0.088 (0.002) | 0.077 | 0.098 | 285 | 0.002 |

Table 2. Morphometric measurement of juveniles newly hatched in the laboratory. Number of individuals (n), average (MEAN), standard error (SE), minimum (MIN), maximum (MAX). See materials and methods for other abbreviations.

| | Live individuals | | | | Preserved individuals | | | |
|----------|------------------|---------------|-------|-------|-----------------------|----------------|-------|-------|
| | n | Mean (1SE) | Min | Max | n | Mean (1SE) | Min | Max |
| TL (mm) | 6 | 43.29 (2.206) | 37.35 | 49.16 | 7 | 42.43 (0.855) | 39.00 | 45.50 |
| SVL (mm) | 6 | 19.78 (0.894) | 16.87 | 22.41 | 7 | 20.23 (0.359) | 18.67 | 21.50 |
| TLL (mm) | 6 | 23.51 (1.364) | 19.31 | 26.75 | 7 | 22.01 (0.384) | 20.50 | 23.56 |
| HEL (mm) | 6 | 4.39 (0.065) | 4.13 | 4.58 | 7 | 4.39 (0.071) | 4.18 | 4.69 |
| SFL (mm) | 6 | 6.81 (0.274) | 5.72 | 7.79 | 7 | 6.98 (0.057) | 6.70 | 7.19 |
| FHL (mm) | 6 | 10.35 (0.965) | 6.91 | 13.50 | 7 | 10.64 (0.355) | 9.09 | 12.05 |
| HH (mm) | 6 | 2.13 (0.034) | 2.05 | 2.29 | 7 | 2.26 (0.081) | 1.90 | 2.47 |
| HW (mm) | 6 | 2.74 (0.051) | 2.55 | 2.89 | 7 | 2.87 (0.043) | 2.69 | 3.01 |
| MASA (g) | 6 | 0.17 (0.015) | 0.10 | 0.20 | 7 | 0.004 (0.0002) | 0.003 | 0.005 |
| HEL/SVL | 6 | 0.224 (0.012) | 0.191 | 0.264 | - | - | - | - |
| SFL/SVL | 6 | 0.346 (0.014) | 0.317 | 0.400 | - | - | - | - |
| FHL/SVL | 6 | 0.518 (0.027) | 0.410 | 0.602 | - | - | - | - |
| HH/SVL | 6 | 0.109 (0.005) | 0.095 | 0.125 | - | - | - | - |
| HW/SVL | 6 | 0.140 (0.008) | 0.120 | 0.171 | - | - | - | - |

Table 3. Morphometric measurements of juvenile individuals caught in the wild. Number of individuals (n), average (MEAN), standard error (SE), minimum (MIN), maximum (MAX). See materials and methods for other abbreviations.

| | Papuk | | | | Ilok |
|----------|-------|---------------|-------|-------|-------|
| | n | Mean (1SE) | Min | Max | |
| TL (mm) | 11 | 52.55 (1.537) | 45.19 | 59.30 | 57.97 |
| SVL (mm) | 20 | 23.74 (0.461) | 19.94 | 26.48 | 27.25 |
| TLL (mm) | 11 | 28.33 (0.935) | 24.46 | 32.92 | 30.72 |
| HEL (mm) | 20 | 4.69 (0.089) | 3.17 | 5.15 | 4.97 |
| SFL (mm) | 20 | 8.11 (0.101) | 7.25 | 8.80 | 7.88 |
| FHL (mm) | 20 | 14.11 (0.326) | 11.83 | 16.38 | 16.84 |
| HH (mm) | 20 | 2.16 (0.051) | 1.93 | 2.90 | 2.18 |
| HW (mm) | 20 | 3.03 (0.062) | 2.13 | 3.57 | 3.08 |
| MASA (g) | 2 | 0.13 (0.010) | 0.12 | 0.14 | - |
| HEL/SVL | 20 | 0.199 (0.005) | 0.143 | 0.248 | 0.182 |
| SFL/SVL | 20 | 0.343 (0.004) | 0.316 | 0.393 | 0.289 |
| FHL/SVL | 20 | 0.594 (0.005) | 0.557 | 0.623 | 0.618 |
| HH/SVL | 20 | 0.092 (0.003) | 0.075 | 0.131 | 0.080 |
| HW/SVL | 20 | 0.128 (0.003) | 0.096 | 0.144 | 0.113 |

significant separation of males and females based on FHL/SVL, SFL/SVL and HL/SVL.

Prevalence of injuries

In Papuk, 34.1 % (43 individuals) of the captured adults had

a tail that had regenerated at least once, or was in the process of regeneration. Two individuals had injuries elsewhere on the body, but complete tail. Out of 20 juvenile individuals from Papuk, six had regenerated and one individual had injured tail (35 %). In Ilok, only three of 11 adult individuals

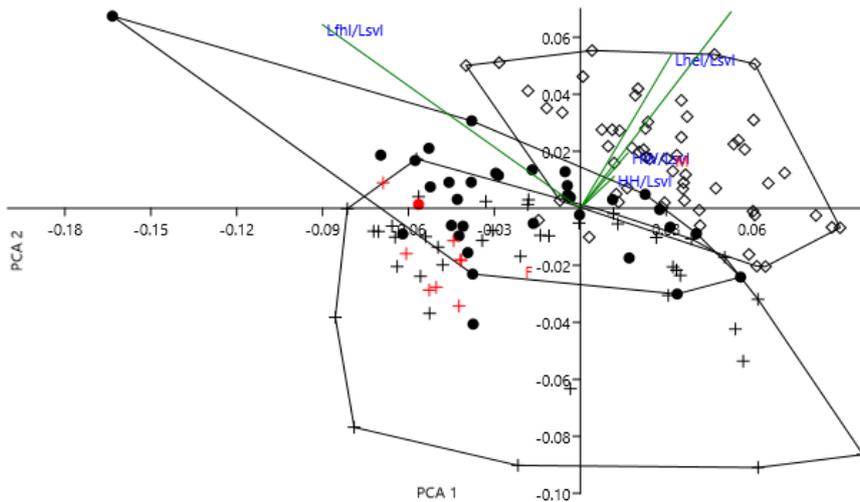


Figure 2. PCA analysis of five morphological indexes (PCA 1 explaining 89 % of variation and PCA 2 10 %). Diamond = Males; Cross = Females (individuals in red were confirmed to be female by autopsy); circles (unknown gender).

Table 4. a) Confusion Matrix of Discriminant analysis done only on indexes (rows are given groups; columns are predicted groups) shows 50.71 % correctly classified; b) Loadings of variables (indexes) in the discriminant analysis.

| a) | Unknown | Females | Males | Total |
|---------|---------|---------|-------|-------|
| Unknown | 15 | 16 | 19 | 50 |
| Females | 10 | 36 | 16 | 62 |
| Males | 6 | 2 | 20 | 28 |
| Total | 31 | 54 | 55 | 140 |

| b) | Axis 1 | Axis 2 |
|-----------|---------|---------|
| LHL/LSVL | 0.0167 | 0.0062 |
| LSFL/LSVL | 0.0246 | 0.0037 |
| LFHL/LSVL | -0.0095 | -0.0188 |
| HH/LSVL | 0.0038 | 0.0057 |
| HW/LSVL | 0.0059 | 0.0086 |

Table 5. Loadings of variables (indexes) in the PCA analysis.

| | PC 1 | PC 2 | PC 3 | PC 4 | PC 5 |
|-----------|--------|--------|---------|--------|---------|
| LHEL/LSVL | 0.2870 | 0.4860 | 0.7920 | 0.2292 | 0.0402 |
| LSFL/LSVL | 0.4745 | 0.6210 | -0.6011 | 0.1669 | 0.0047 |
| LFHL/LSVL | 0.8116 | 0.5806 | 0.0508 | 0.0365 | 0.0175 |
| HH/LSVL | 0.1067 | 0.1010 | 0.0723 | 0.4431 | 0.8814 |
| HW/LSVL | 0.1498 | 0.1755 | 0.0600 | 0.8497 | -0.4703 |

(27.3 %) had previously lost their tail.

Sexual dimorphism

Gender in Snake-eyed Skink could not always be determined by probing or popping due to its small size. If done with too much force, both methods could lead to an injury of the individuals. In that context, sex was confirmed only in nine individuals from Papuk, by inspection of gonads, seven females and two males. On the individual inspection, we determined another 12 individuals from Papuk as gravid females. One individual from Ilok was determined as gravid based on the bite marks. Minimal and average SVL and FHL of sexually matured females are higher than the average measurements of the whole examined samples. The biggest individual recorded was a female caught in Papuk (SVL = 62.64). Minimal and average BM of female individuals after the egg deposition were higher than the average BM, which suggests that sexually mature females are generally larger and could probably be identified based on their size.

Reproduction

Of 21 female that were taken for the reproduction study (seven in 2010, two in 2011 and 12 in 2012), only nine individuals deposited eggs. The other females were probably not gravid. In the females *post partum*, the folds on the skin were not observed, and the only difference was that they were much thinner.

In seven females that were sacrificed after the eggs deposition, the biggest observed follicle was 1.37 mm, and in one individual, there were no follicles in its ovaries. Generally, size of the follicles in both ovaries suggests that none of the females examined would have another clutch in the same season (on average 1 (1SD: 0.315) bigger follicles in the left ovary, mean size of 0.83 mm; 2 (1SD: 0.397) bigger follicles in the right ovary, mean size of 0.86 mm; and few smaller follicles in each of the ovaries).

The observed clutch size in females spanned between 2-4 eggs with an average of 2.78 eggs. Out of nine clutches, five had 3 eggs, three clutches had 2 eggs and only one clutch had 4 eggs. Out of the 25 eggs that were deposited, 9 eggs (36 %) did not develop and after they were opened and inspected, they were marked as not fertilized as there were no visible signs of a development of an embryo. From the remaining 16 eggs, two eggs failed to hatch and after the inspection of those two eggs, it was observed that there were possible malformations in the lumbar spine and the lower jaw.

On average, females had 1.56 young with almost 50 % of the energy invested in offspring being lost up to this stage. Comparison of egg dimensions, volume and mass of both fertilized and non-fertilized eggs did not show significant difference between them (Mann-Whitney U test; $p > 0.05$; Table 6). Fertilized eggs have bigger mass and continue to grow through incubation.

Egg growth

Incubation time was determined only for two clutches and lasted 34 days for all six eggs. Monitoring of egg sizes showed that average length, width, mass, surface and volume increase linearly during the incubation time (Fig. 3). The ratio of the initial average length and width is a bit lower than later during the incubation (allometric growth), which shows that the eggs are more elongated at the begin

Table 6. Measurements of females prior and post oviposition and size of the freshly deposited eggs (including non-fertilised eggs). m_1 – female mass prior to oviposition. m_2 – female mass after oviposition. RCM – relative clutch mass (ratio of clutch mass to maternal mass after oviposition).

| | n | Mean (1SE) | Min | Max |
|--|----|-----------------|--------|--------|
| SVL female (mm) | 9 | 49.05 (0.637) | 46.68 | 53.08 |
| m_1 (g) | 9 | 1.56 (0.094) | 1.20 | 2.00 |
| m_2 (g) | 5 | 1.26 (0.075) | 1.07 | 1.46 |
| Clutch size | 9 | 2.78 (0.133) | 2 | 4 |
| Clutch volume (mm ³) | 9 | 411.32 (36.883) | 322.68 | 616.77 |
| Clutch mass (g) | 8 | 0.53 (0.056) | 0.37 | 0.84 |
| RCM (g) | 8 | 0.33 (0.036) | 0.25 | 0.50 |
| Egg mass ₀ (g) | 22 | 0.19 (0.005) | 0.16 | 0.26 |
| Egg length ₀ (mm) | 25 | 10.00 (0.146) | 8.71 | 11.83 |
| Egg width ₀ (mm) | 25 | 5.29 (0.084) | 4.65 | 6.07 |
| Egg volume ₀ (mm ³) | 25 | 148.08 (5.835) | 100.20 | 196.22 |
| Egg surface (mm ²) | 25 | 143.64 (3.698) | 111.27 | 171.96 |
| Juvenile mass (g) | 6 | 0.17 (0.015) | 0.10 | 0.20 |

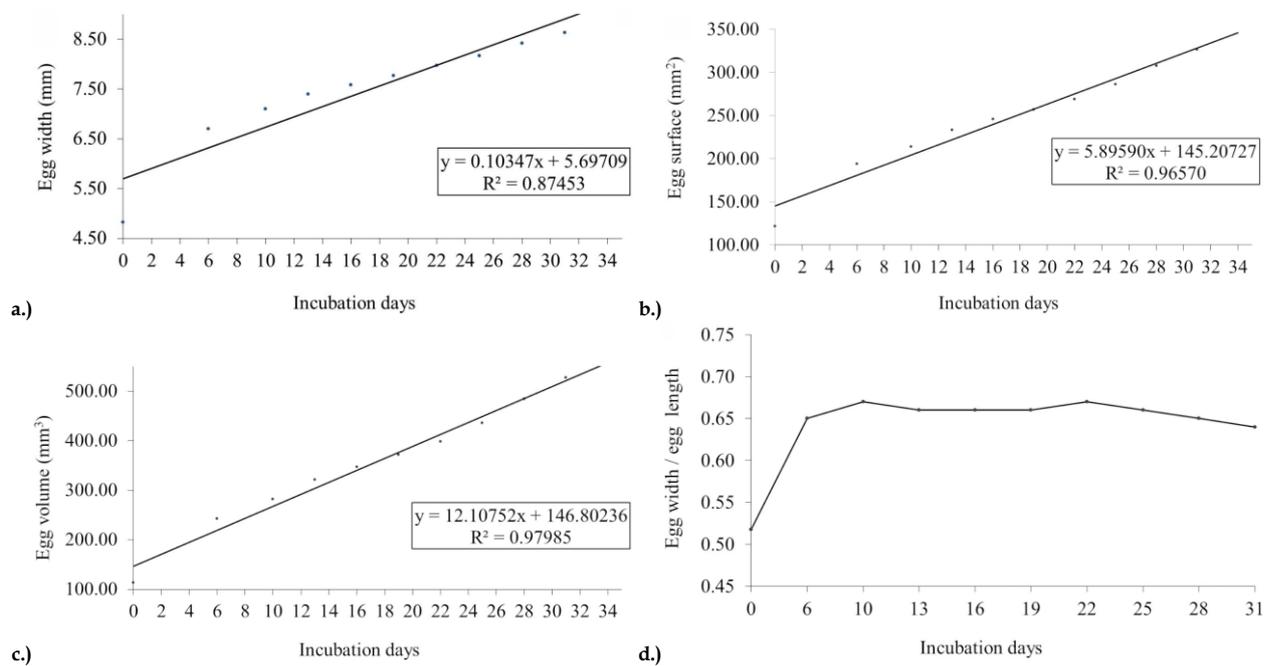


Figure 3. Egg development in time (n=6): a) egg width, b) egg surface, c) egg volume, d) ratio egg width/ egg length.

Table 7. Comparison of mass (m_0), volume (V_0), length (L_0), and width (W_0) of fertilized and non-fertilized eggs.

| | Fertilized eggs | | | Non-fertilized eggs | | | Mann-Whitney U | | | |
|--------------------------|-----------------|----------------|--------|---------------------|---|----------------|----------------|--------|------|-------|
| | n | Mean (1SE) | Min | Max | n | Mean (1SE) | Min | Max | U | p |
| m_0 (g) | 16 | 0.19 (0.007) | 0.16 | 0.26 | 6 | 0.20 (0.008) | 0.16 | 0.21 | 32 | 0.261 |
| V_0 (mm ³) | 16 | 148.92 (7.949) | 100.20 | 196.22 | 9 | 146.57 (8.524) | 110.33 | 181.19 | 68 | 0.846 |
| L_0 (mm) | 16 | 9.84 (0.145) | 8.71 | 10.75 | 9 | 10.30 (0.301) | 9.01 | 11.83 | 50 | 0.229 |
| W_0 (mm) | 16 | 5.34 (0.118) | 4.65 | 6.07 | 9 | 5.20 (0.108) | 4.76 | 5.65 | 60.5 | 0.522 |

ning, and the same happens at the end of the incubation.

Correlating the size of females and their clutches

Since there were no significant differences in the sizes of fertilized and non-fertilized eggs (Table 7), all eggs were included in the analysis. Female BM after egg deposition and egg mass showed slight negative correlation, but this was not significant ($r = -0.25$; $p = 0.406$). Female SVL and clutch size did not show any correlations ($r = 0.136$; $p = 0.727$), similar to FHL and clutch size ($r = 0.125$; $p = 0.749$). Female BM and clutch size showed some positive correlation, however it

was also not significant ($r = 0.846$; $p = 0.071$), similar to female SVL and clutch volume ($r = 0.519$; $p = 0.152$), and female FHL and clutch volume ($r = 0.645$; $p = 0.060$).

However, SVL and FHL both showed positive, significant correlation with the volume of each egg (SVL $r = 0.496$; $p = 0.012$; FHL $r = 0.624$; $p = 0.001$; Fig. 4 and Fig. 5). These results suggest that the increase of the size of the female does not result in an increased number of eggs, but in an increase of the size of each individual egg. Egg volume and SVL of hatchlings were correlated, but possibly due to the small sample size, this correlation was not significant ($r =$

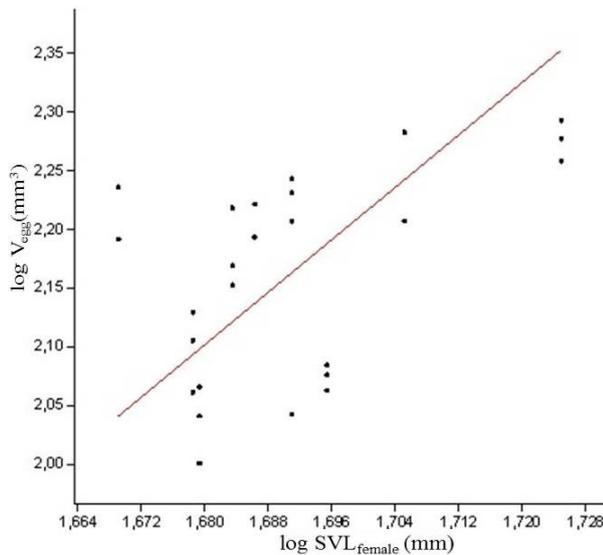


Figure 4. Female size (SVL) and egg volume (V) correlation. Data was log transformed.

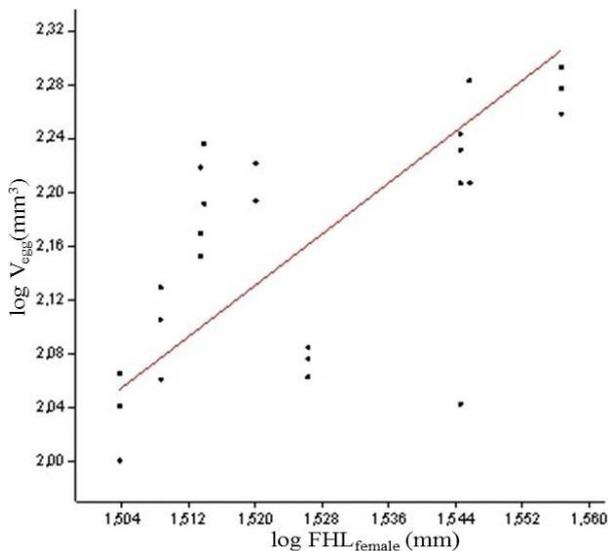


Figure 5. Female FHL and egg volume (V) correlation. Data was log transformed.

0.734; $p = 0.097$).

Discussion

Data on the Snake-eyed Skink morphology obtained in our research showed some differences between the two studied populations. Individuals from Ilok were on average bigger (TL, TLL, SVL, FHL, relative FHL) and heavier than individuals from Papuk, while on the other hand, individuals from Papuk had on average greater head proportions (HEL, HH, HW, SFL). Higher SVL and FHL values were recorded in females, and this could be expected considering their body constitution, since a longer body would provide more space for eggs, which is important for fecundity and would be selected for. This could explain larger average body size in Ilok population, since there females were more commonly examined than males. However, in order to confirm this, a

greater sample size from Ilok would be needed.

Furthermore, we confirmed that body length alone is not sufficient to determine the age group of Snake-eyed Skink, and it can be used only as a rough guide, and the only reliable method is by inspecting the gonads of each individual to determine if they are sexually mature.

Sex determination in Snake-eyed Skink in the field is a challenging task since none of the commonly used methods (e.g. extrusion of hemipenises) showed to be effective with this species. Generally, the only reliable method for sex determination and determination of sexual maturity in species without distinct sexual dimorphism is by inspection of gonads. Due to its invasiveness, we can rule out this method as an option for larger number of individuals. In that context, the reliable field method for sex determination in the Snake-eyed Skink still needs to be further developed, and our research might be a step towards this goal. When examining the morphometric of females and males, on the graphs we can see some differences. However, due to our small sample size, we could not determine it thoroughly, but these results coincide with the observations from others, e.g. Gruber (1981), Schmidler (1997), Ljubisavljević et al. (2002). Nevertheless, these differences are not very pronounced, which allows, for example, subadult females to be misidentified as males, so this method can only be used as a rough estimations.

In juvenile individuals, greater head proportions to body size were expected, since this is common in many species, such as in lacertid lizards (Kirchhof et al. 2012), where allometric growth is frequently observed; this was also confirmed in our case. Larger heads allow the animals to feed on relatively bigger prey in order to provide more energy. Gruber (1981) and Arnold (2002) observed that juvenile individuals of Snake-eyed Skink mostly rely on their limbs when moving, while as they grow, they gradually change to more snake-like movement, which is also confirmed with the increase of relative body length with age.

Juvenile individuals have insignificantly a higher number of injuries than the adults, but since smaller animals are easier to fight down, some of them do not survive predator's attacks, so the total percentage of assault on juveniles is probably much higher than the recorded 35 %. The total number of juvenile records in our study was much lower than of adults, which result of lower detection rate and higher predation pressure on juveniles, and this was confirmed by injuries prevalence data. Since this is a relatively high percentage, it adds to the importance of bright tail colouration in juveniles for the distraction of predators. On the other hand, adult individuals are bigger, adroit and more experienced in escaping predators. However, probably the total rate of injuries may even be higher than the one observed, as it seems that not all regenerated tails were identified as such (based on their tail length compared to that of other individuals with intact tails). In Ilok, total percentage of the individuals with regenerated tails was lower than in Papuk population, and this could be a consequence of reduced predation risk due to the higher anthropogenic influence.

Gruber (1981) describes the Snake-eyed Skink as a non-territorial species, which rules out the injuries from its conspecific males. On the other hand, Fejérváry (1912) affirms

that male to male fights precede the mating, but during this research, we could not confirm this.

Although territoriality could explain larger head proportions in males, according to Kirchoff et al. (2012) bigger heads can also be advantageous while holding the female during mating. This could explain the minor differences in head proportions, since in territorial species, these differences are much more pronounced.

Several authors have reported bright colouration on the belly in males during the breeding season in closely related species, e.g. *A. chernovi* (Eiselt 1976, Gruber 1981, Göçmen et al. 1996, Kumlutas et al. 2005, Schmidler 1997) and *A. budaki* (Schmidler, 1997), and Ljubisavljević et al. (2002) accounted for similar phenomenon in the hybrid population of *A. kitaibelii fitzingeri* × *stepaneki*. However, in our research we observed not only males, but also gravid females with the same bright colouration. Göçmen et al. (1996) had similar observations in *A. chernovi*, even if more commonly in males. This would suggest the colouration to be a sign of sexual activity, and not sex-related, so this trait cannot be used in sex determination.

During our research we failed to find subadult individuals, and possible reason could be their reduced activities level. We can presume them to be more cautious than the newly hatched individuals, among others, since they do not have the juvenile bright tail colouration. Subadults are probably also less active than the adults since adults spend more time searching for partners.

The Snake-eyed Skink reaches sexual maturity approximately at the age of two, and lives up to 3.5 years (Gruber 1981). This is opposing the theory that short-lived animals reach sexual maturity sooner and commonly have more than one clutch per year in order to maximize the number of offspring during their life span (Adamopoulou & Valakos 2000). We observed that individuals that hatched earlier (e.g. in August), lose their bright colouration before hibernation and are probably sexually mature the following spring, and our observations of juveniles from October with reduced red colouration support this idea. On the other hand, those individuals that hatched later (i.e. October) will lose their colouration only later in the spring and would mate in their second year of life. After mating (April-May) and egg deposition (June till mid-August), hatching occurs from August throughout September (Fejervary 1912, Gruber 1981). The earliest hatching in the Snake-eyed Skink was reported from Slovakia from the end of June (Korsós et al. 2008), but possibly as a consequence of the extremely warm season. Since neither in June nor July we could find newly hatched juveniles, and no vitellogeneous follicles in examined female gonads, we can conclude that the Snake-eyed Skink has only one brood per season, opposite to our expectations of at least two broods. Clutch size of our studied population is consistent with the literature data (2-4 eggs per clutch; Gruber 1981, Arnold 2002); however, Gruber (1981) found on average four eggs per clutch in *A. k. fitzingeri* and *A. k. stepaneki*, and in our case the average were 2.78 eggs per clutch.

During the eggs development, one could expect them to show linear growth of dimensions and mass during the incubation, since this phenomenon has been observed in 64 European lacertid species (Bosch & Bout 1998), and it was also confirmed in our case. Generally in reptiles, the eggs of

more elongated species are more elongated in order to be able to pass through the females' pelvis (Pritchard 1979, Elgar & Heaphy 1989).

In many lizard species, clutch size increases with the increase of the female body size, but this correlation is commonly not observed in species with small clutch size (Polović et al. 2013). Generally, females can have either fewer larger eggs or more smaller eggs. In the Snake-eyed Skink the selection favoured the size of the eggs, over the quantity. We observed stronger correlation between the eggs' volume and female size, than between eggs' number and female size. Olsson & Shine (1997) examined the influence of the two possible causes for this, and concluded that resource availability is the proximal cause, but the volume of the available space in the females' body is also important. Although we found slight correlation between the egg volume and the size of a hatchling, it was not significant and this could be due to the small sample size. The small number of eggs per clutch is compensated by the bigger size of the hatchlings, which gives them advantages in survival in comparison to smaller hatchlings (Adamopoulou & Valakos 2000).

Acknowledgements. The authors would like to thank all of the members of Croatian Herpetological Society HYLA that participated in this long term research, and the staff of Public Institution Nature Park "Papuk" who recognized the value of this project and financially supported it. Especially, we would like to acknowledge the great contributions of Frano Barišić, Senka Baškiera, Sven Kapelj and Ivan Damjanović. We would like to thank the Croatian Ministry of Environmental and Nature Protection for issuing the research permit UP/I-012-07/10-33/733 and UP/I-612-07/14-45/01.

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