Ecology of the small Snake-eyed Skink *Ablepharus kitaibelii* (Bibron & Bory de Saint-Vincent, 1833) in a NW Bulgarian population

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Abstract. The Snake-eyed Skink *Ablepharus kitaibelii* (Bibron & Bory de Saint-Vincent, 1833) is one of the smallest lizards in Europe, and this, combined with its secretive lifestyle, makes population studies on the species very difficult. Only a few studies concerning sparse data, mainly on the species' activity, have been published, but its population ecology has received less attention. We aimed to collect information about population parameters such as population size, survival rate, Body Condition Index (BCI), and individual movements. Here we present data from a four-year Capture–Mark–Recapture study on a population of *A. kitaibelii* from Northwestern Bulgaria. We marked 415 individuals (136 females, 108 males, and 171 juveniles). Rapid sexual maturation and extended egg-laying period led to an overlapping of the generations in time – in many individuals, the onset of sexual maturity overlaps with the period of the next hatchlings – probably crucial for the survival of the species. The Cormack–Jolly–Seber model estimated that the average adult population size for the study period was N = 220 (95%CI: 102–490). Long-term recaptures suggest that it is generally sedentary, likely without performing many long-distance movements more than 60 m and usually maintaining a small individual range. A low and uneven recapture rate by season was due to the secretive lifestyle of the species and seasonal changes in habitat characteristics such as overgrowing with grasses and/or bushes.

Keywords: capture-mark-recapture, demographics, generation, Scincidae, survival.

Introduction

The Snake-eyed Skink, *Ablepharus kitaibelii* (Bibron & Bory de Saint-Vincent, 1833), distributed from southernmost Slovakia and reaching the southern edge of the Balkan Peninsula in Greece (Gruber 1981), is the only representative of the family Scincidae in Bulgaria. The species has a patchy distribution across the country. It inhabits dry meadows and sparse oak forests mainly in lowlands up to medium elevations in mountainous areas, typically up to 1000 m a.s.l. Its distribution in the lowland areas, especially in Northern Bulgaria, is limited to fragmented relict populations due to deforestation. During the hot summer months, the species predominantly hides under fallen leaves and stones, but remains highly secretive throughout its active period (Beshkov & Nanev 2006, Stojanov et al. 2011).

Although some ecological information on the activity and reproduction of *A. kitaibelii* in Bulgaria was published recently (Vergilov & Natchev 2018, Vergilov et al. 2018a, b), data on its population parameters are largely lacking. This limits future scientific studies and evidence-based conservation practices.

Capture–Mark–Recapture (CMR) is a robust method for collecting population estimation data. It is used widely in population studies on amphibians and reptiles (Henderson 2003, Rockwood 2006, Sutherland 2006). CMR has been applied to different Scincidae species (Towns 1975, Henle 1989, Pitt 2001, Bloomberg & Shine 2001, Sumner et al. 2001, Akani et al. 2002, Stow & Sunnucks 2004, Chapple & Keogh 2006, Lettink et al. 2011, Dubey et al. 2013, Hoehn et al. 2015). Permanent marking as part of CMR has been performed previously on lizards (e.g., using heat-branding techniques that burn off a scale: Stumpel 1985, Ferreiro & Galán 2004, Vervust & Van Damme 2009, Ehmann 2000,

Hitchmough et al. 2012); the most widely used markings have been practiced for a long time, and they do not induce long-lasting negative effects to individuals.

So far in Bulgaria, few publications used CMR for amphibians (Beshkov 1972, Beshkov & Jameson 1980, Beshkov & Angelova 1981, Beshkov et al. 1986, Naumov et al. 2020, Lukanov et al. 2021, Lukanov in press) and only two for a single viperid species (Dyugmedzhiev et al. 2018, 2020). We used heat-branding using a portable soldering iron to mark the Snake-eyed Skinks individually (Vergilov & Tzankov 2018).

The present study aims to fill knowledge gaps on the ecological characteristics of the small and secretive Snake-eyed Skink, utilizing the CMR to obtain population parameters (size, individual survival rate) and individual movements.

Material and Methods

The study area was located on Pastrina Hill (almost entirely within the Natura 2000 Site of Community Importance "Pastrina"), a low elevation (563 m) continental climate site in northwestern Bulgaria (Fig. 1; see Vergilov & Natchev 2017). We concentrated our search efforts on the ecotone zone between an oak forest and a meadow because it is among the species' most suitable habitats (Fejérváry 1912, Fuhn 1970, Gruber 1981, Herczeg et al. 2007, Stojanov et al. 2011, Vacheva et al. 2020). The small size of the study area (app. 3 ha) corresponded to the size of the species and its presumed limited mobility. The study area was visited for six to seven days per month in May (spring), July (summer), and September (autumn) for four years (2013–2016). The active searching was approximately 3 hours per visit; over 95% of the time, only one person (VV) was actively catching and searching for the lizards. May and September fall into the peak of the active period for the species when individuals move above ground; July was sampled to obtain life history data of lizards
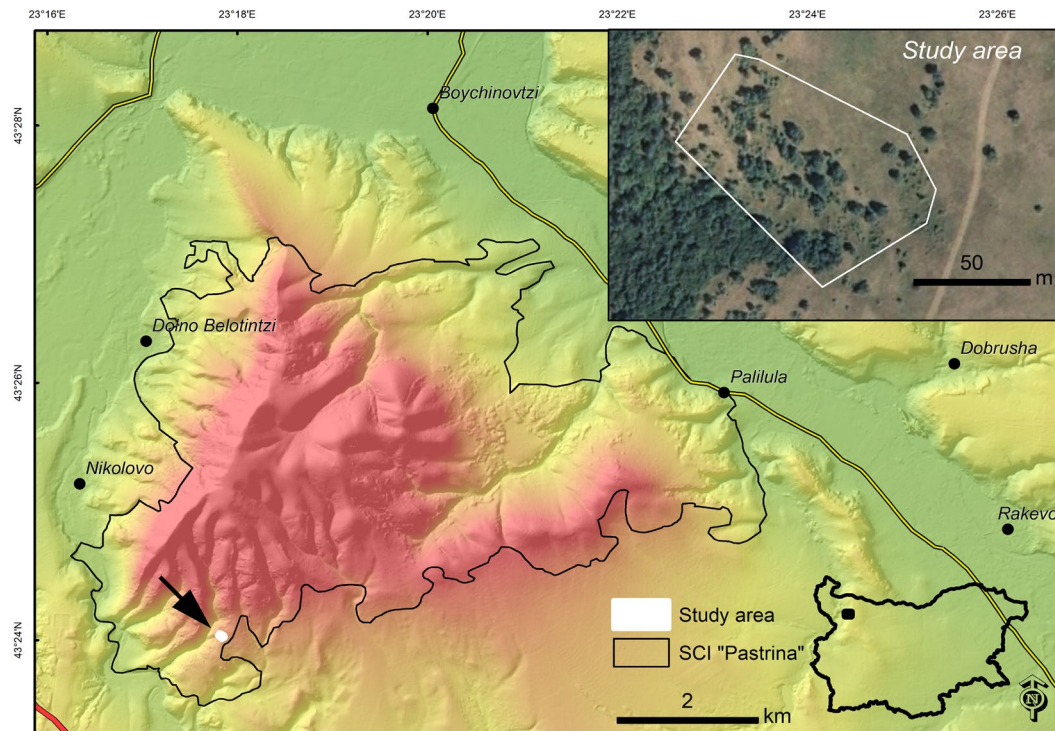
Figure 1. The study area (bottom left) in Pastrina Hill and the SCI "Pastrina", Bulgaria. Inset: a satellite image of the study area showing the ecotone.

when they spent less time visible above ground and instead hide under fallen foliage due to the elevated environmental temperatures.

We used CMR, capturing individuals by hand after detecting them visually.

The following data were taken for each individual at (re)capture: individual code, capture location (using a hand-held GPS Garmin Map62s, accuracy: 3–5 m), body length (SVL, from the rostrum to the end of the cloacal scales, using a plastic ruler, to the nearest mm), tail length (TL, from the end of the cloacal scales to the tip of the tail), weight (W, measured with a small portable electronic scale My Weight DuraScale D2 300, accuracy ± 0.01 g), and sex (determined visually by habitus for adults) (see Ljubisavljević et al. 2002). Based on the skeletochronology and the histology of the gonads (Vergilov et al. 2018a, b), individuals with SVL less than 38 mm were considered juveniles.

When captured, lizards were marked individually by heat-branding with a unique number via a portable soldering iron on batteries, following a new coding system applicable for small squamates (see Vergilov & Tzankov 2018, and references therein). Each individual was photographed with a high-resolution digital camera from the dorsal, ventral, and in some cases, lateral sides. Individual recognition of marked individuals was based on the brand marks and was compared to the photographic material, the size, weight, and coloration of the lizards. Photographic and morphometric verification was especially helpful for old markings that had faded or merged because of growth (Vergilov & Tzankov 2018).

For population parameters estimations, we used only data on adult individuals (SVL ≥ 38 mm; at least 11–12 months after hatching), grouped by sampling month for each year of the study (12 capture-recapture sessions); the recapture rates of juveniles were insufficient to provide usable results. Estimation of the survival rate (phi), capture probability (p), and the population size (N) were performed in MARK 8.1 (White & Burnham 1999), using the Cormack–Jolly–Seber (CJS) (Lebreton et al. 1992) model for open populations.

To estimate long-term movements and individual range, we used all capture and recapture data without grouping them by age or sex. We used the Geospatial Modelling Environment 0.7.4 extension in ArcGIS 10.3 (ESRI, Redlands, California) to obtain the distances between consecutive recaptures.

For morphometric analyses, we used only data from the first capture of an individual to avoid pseudoreplication. Boxplots of SVL and weights of individuals (both adults and juveniles) of the populations by seasons were created with SigmaPlot 12.0 (Systat Software, San Jose, CA). Body Condition Index (BCI) was estimated using a logarithmic regression between weight and SVL (Jakob et al. 1996, Rodríguez-Prieto et al. 2010). Statistical analyses were performed with STATISTICA 10.0 (StatSoft. Inc. 2011,). The statistical significance level was set at 0.05.

Results and Discussion

We marked 415 individuals (136 females, 108 males, and 171 juveniles). To estimate population parameters, we obtained 165 recaptures between seasons (86 for females, 69 for males, and 10 for juveniles). Capture rates were uneven across seasons in each year of the study (Fig. 2), which corresponds to the differential seasonal activity of the species. The potential for bias in (re)capturing individuals due to the chosen method also must be considered.

Despite the unequal number of individuals captured per season (Fig. 2), some major trends can be noted. Although adult individuals were captured throughout the year, most (re)captures were during the spring, when the animals are supposed to be more active (Herczeg et al. 2007, Stojanov et al. 2011, Vergilov 2017) and mating and fertilization take place (Vergilov et al. 2018b). Juveniles were captured mainly in the autumn; those captured in the spring were individuals
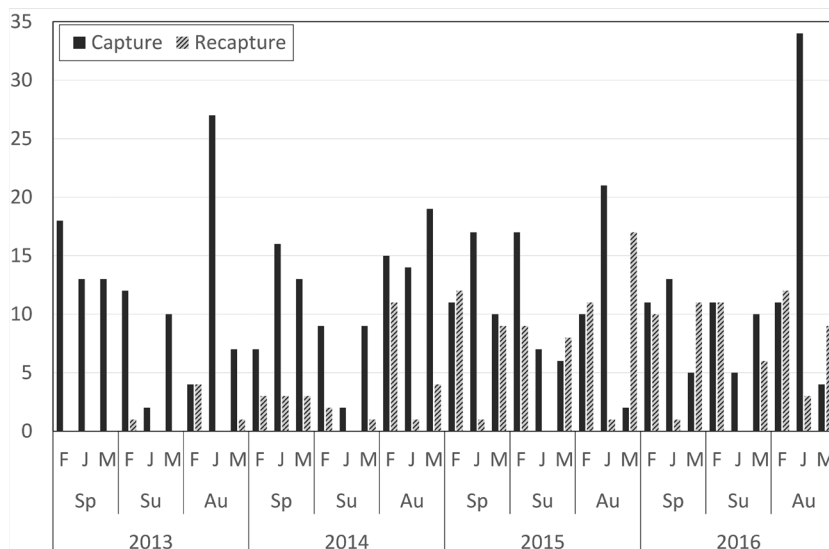
Figure 2. Number of captured and recaptured female (F), male (M), and juvenile (J) individuals of *A. kitaibelii* for four years' active seasons (Sp – spring, Su – summer, Au – autumn).

that hatched the previous year and had not grown enough to become sexually mature. The low number or complete absence of juveniles in the summer is explained by the fact that juveniles from the spring have become sexually mature, while the hatching of the next generation of Snake-eyed Skinks has not yet occurred. During the autumn of 2014, fewer juveniles were captured and observed than in the other years (2013: 27 captured, 0 recaptured; 2014: 14 captured, 1 recaptured; 2015: 21 captured, 1 recaptured; 2016: 34 captured, 3 recaptured). We speculate that the unusually large amount of precipitation in the summer of 2014 compared to the other years resulted in considerable moisture within the soil during the egg laying period and increased embryonic mortality. Furthermore, *A. kitaibelii* generally inhabits dry and warm habitats, such as sparse oak forests, with soil that is not exposed to prolonged water saturation while retaining sufficient soil moisture to prevent eggs from drying out (Vergilov & Popgeorgiev, pers. obs.).

Females were significantly larger than males (for females – range: 39.00–54.30 (mean = 46.27 ± SD = 3.55) mm, n = 136; for males: 38.00–49.00 (42.59 ± 2.33) mm, n = 108; one-way ANOVA: $p < 0.001$, $F = 899.21$). Females were also heavier than males (for females – range: 0.55–1.72 (mean = 1.04 ± SD = 0.23) g, n = 136; for males: 0.51–1.53 (0.99 ± 0.18) g, n = 108; one-way ANOVA: $p < 0.001$, $F = 642.47$). Captured juveniles had mean SVL = 29.7 mm (range: 21.5–37.8 mm, SD: 4.22, n = 169) and a mean weight of 0.34 g (range: 0.12–0.80 g, SD: 0.16, n = 169).

On recapture, individuals had usually grown since the last capture, i.e., the data on the growth and skeletochronology (Vergilov et al. 2018a) confirmed the results from the population study. The largest female had SVL of 56 mm at last recapture.

Vergilov & Natchev (2018) suggest that juveniles between 0.09 and 0.18 g were captured shortly (within a week) after the hatching. The earliest juvenile individual within this weight interval was caught on 26.07.2015 (0.13 g), and the latest – on 2.10.2015 (0.18 g). This confirms the results of Vergilov et al. (2018b) about the extended hatching period. The smallest newly hatched individual, after incubation, had SVL of 19 mm (Vergilov & Natchev 2018),

and the smallest caught on Pastrina Hill was 21.5 mm. Rotter (1962) stated that the newly hatched juveniles of *A. kitaibelii* had SVL of 33 mm, which greatly differs from Vergilov & Natchev (2018), Vergilov et al. (2018b) and this study.

Most of the largest females (50 mm and more; n = 26) were initially captured in the spring (n = 15) (Fig. 3); during the summer, we captured only eight, and in the autumn – three. This suggests that they likely reach such sizes at the end of their life, and many probably die as the active season progresses. Although males grow slower than females (Vergilov et al. 2018a), their growth continues throughout their life. The largest females and males lived up to four years (Vergilov et al. 2018a), although some individuals may live for some time after the fourth hibernation. The largest individuals were generally the heaviest (Figs. 4, 5).

Correlations (Fig. 5) for females were: spring $r = 0.83$, $p < 0.001$, $y = -1.16 + 0.05x$; summer r = 0.40, $p < 0.005$, $y = -0.08 + 0.02x$; autumn $r = 0.46$, p < 0.005, $y = -0.33 + 0.03x$; for males were: spring $r = 0.67$, $p < 0.001$, $y = -0.97 + 0.05x$; summer r = 0.64, p < 0.001, $y = -1.21 + 0.05x$; autumn r = 0.47, p < 0.01, $y = -0.59 + 0.04x$. Males had less variation in the BCI than females, overall and across seasons (Figs. 4, 5), likely due to the faster growth and egg deposition in females' abdomens (Vergilov et al. 2018a). Moreover, the BCI of the males was slightly higher in summer, probably due to the lower energy expenditure during the reproductive period. Still, BCI values should be treated with caution as BCI can be an unreliable indicator of fitness (Dudek et al. 2015).

When comparing the SVL of juveniles across different seasons (Fig. 3), we measured newly hatched ones with SVL of 20–24 mm in the summer and autumn, comparable to the 18–22 mm reported in Vergilov & Natchev (2018). Also, there were individuals with SVL of 37–39 mm in the spring and summer. Individuals with SVL of 39 mm had undergone two hibernations (Vergilov et al. 2018a), which is also the SVL of sexual maturation (Vergilov et al. 2018b). Furthermore, the presence of immature individuals in July suggests that they probably hatched later in the previous year (in the autumn; op. cit.). Comparison between the histological studies and the population study reveals an
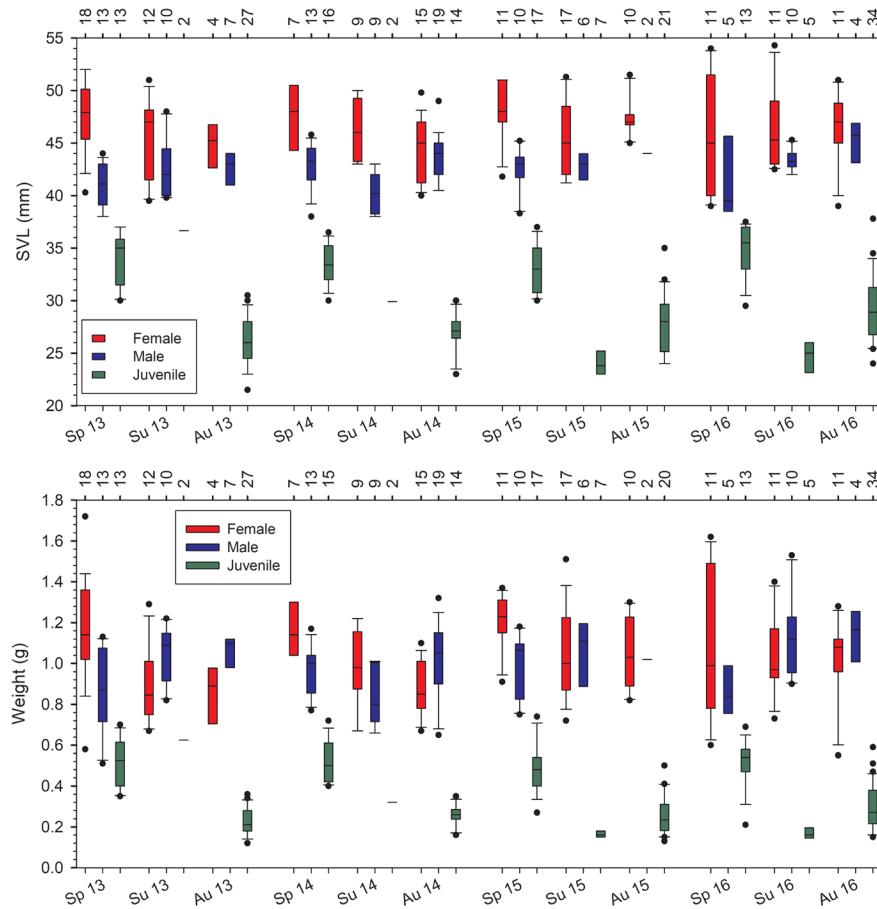
Figure 3. Body length (SVL, top) and weight (W, bottom) of adult females, adult males, and juveniles of *A. kitaibelii* at first capture in spring (Sp), summer (Su), and autumn (Au) in 2013–2016. Numbers on the top x-axis are respective sample sizes.
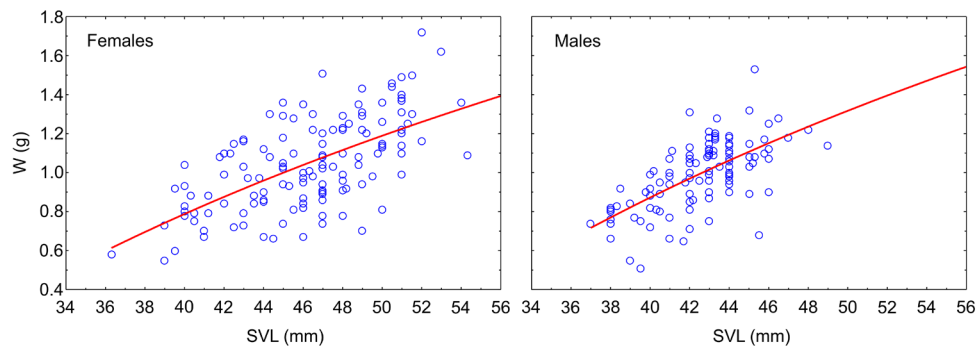

Figure 4. Body Condition Index (BCI) of females (left) and males (right) of *A. kitaibelii*. Dashed lines are 95% confidence intervals. Correlation for females was $r = 0.63$, $p < 0.001$, $y = -0.79 + 0.04x$; for males was $r = 0.63$, $p < 0.001$, $y = -1.00 + 0.05x$.
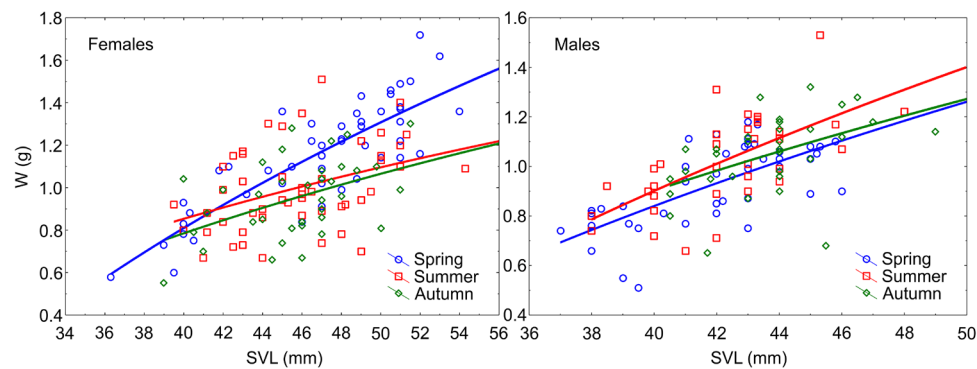

Figure 5. Body Condition Index (BCI) of females (left) and males (right) of *A. kitaibelii* in spring, summer and autumn.

overlapping of the generations in time – in many individuals, the onset of sexual maturity overlaps with the period of the next hatchlings in the summer and autumn seasons. The incubation period is about one month, and the egg-laying period is extended (Vergilov et al. 2018b). Based on the data of this study and the data from the histological studies (Vergilov et al. 2018a, b), we can conclude that the individuals that hatch earlier (at the beginning or the middle of July) have more time to grow before and after the first hibernation, i.e., they could become sexually mature at a body length of 38–39 mm in the spring, after their first hibernation. On the other hand, individuals that hatch later (late September) have less time to grow up to 39 mm. They could become sexually mature much later after the first hibernation or after the second hibernation, especially considering that the growth rate slows before hibernation. This overlapping of generations is probably one of the main strategies for the species' survival.

We generated 16 models within CJS. Based on the ΔAIC, we chose the second-best model due to its low Akaike's Information Criterion (Akaike 1973) that also resulted in the smallest confidence intervals and represented the most feasible results. This model was based on a constant capture probability (p) and variable survival rate over time {$phi(t)$} (AIC = 839.56; ΔAIC = 13.28). The model with the lowest ΔAIC was {$phi(t)$ $p(g*t)$} (AIC = 834.62; ΔAIC = 8.34), and the third model was {$phi(t)$ $p(g)$} (AIC = 841.59; ΔAIC = 15.32).

The Cormack-Jolly-Seber model estimated that the average adult population size (N) for the study period was N = 220 (95%CI: 102–490). The estimated N matches the number of marked adults (n = 265), considering the population is open, and during the study duration there were mortality and recruitment/transition of juveniles to adults, as well as likely emigration and immigration. We excluded juveniles from the estimations because they highly increased the variance in the confidence intervals due to their low recapture rate. The low p-value (mean = 0.346; 95%CI = 0.270–0.432) is most likely due to difficulties capturing the small agile individuals, seasonal changes in microhabitat conditions such as the growth of grasses, and changes in the seasonal activity of individuals linked to

increased secretive lifestyle. The high phi value (mean = 0.995; 95%CI = 0.987–0.998) suggests that a more accurate estimation of survivability requires surveying for a longer period.

We estimated movements based on 242 consecutive capture/recaptures of 132 individuals. Our results suggest that *A. kitaibelii* is highly sedentary, with most recaptures < 10 m away from each other (Table 1; Appendix 1). Calculations of home ranges and exact distances moved are not feasible, given the method of recapture and the intrinsic error of the hand-held GPS that may be as low as ± 5 m under best conditions – 38% of the distances are < 5 m. This compromises comparison between age, sex, or seasonality and statistical analyses.

Table 1. Frequency of distances moved by *A. kitaibelii*, in 10-m bins. Included are all recaptures, including within the same capture sessions and seasons.

Range (m)	Frequency	Cumulative %
0–10	180	74.38%
10–20	39	90.50%
20–30	10	94.63%
30–40	9	98.35%
40–50	1	98.76%
50–60	3	100.00%

However, a few interesting observations lend further support to the sedentary lifestyle of *A. kitaibelii*. Overall, we have 18 capture-recaptures more than 365 days apart, with an average distance of 13.3 m (range: 3.1–39.0 m) between original and subsequent locations. The longest period between a recapture of an individual was 1105 days (6 May 2013 to 15 May 2016); the adult male was located 6.4 m away from the original capture location. Furthermore, the four individuals with the most recaptures (11, 8, 7, and 6) all seem to have stayed within small individual ranges (Fig. 6); three of them had made single long-distance dispersals (ID 154: 21.9 m, ID 74: 32.7 m, ID 241: 53.5 m), but then returned almost exactly to the previous capture location. There are no
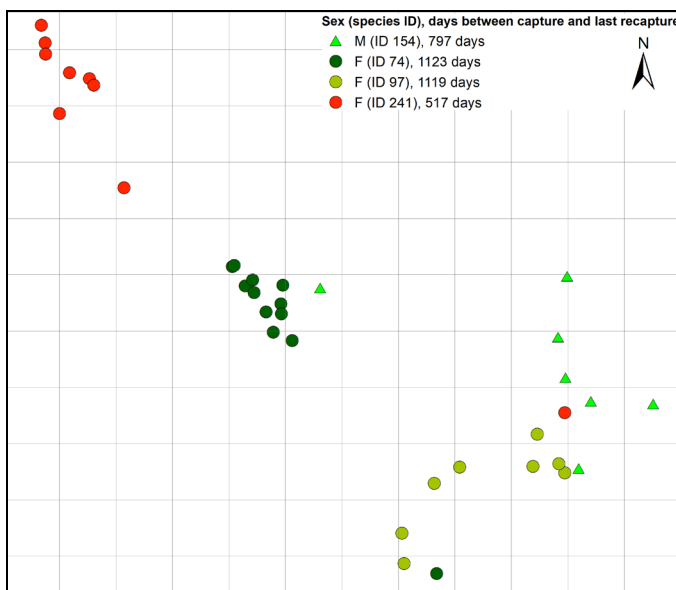

Figure. 6. Capture/recapture locations of the four *A. kitaibelii* with the highest number of recaptures. Squares = 5 × 5 m.

published data supporting territoriality in this species, although there are some sparse data concerning interspecific aggression (Teschler 1885, Vergilov & Kornilev 2019), likely due to the presumably small and overlapping home ranges of the Snake-eyed Skink's individuals.

For future CMR studies on the Snake-eyed Skink or similar species, finding means to increase the recapture rate will yield more robust estimates. Initially, we planned to capture individuals by hand and with non-lethal pitfall traps (Enge 2001, Sutherland 2006). The traps were plastic cylinders approx. 120 mm high and 100–120 mm in diameter, with a perforated bottom to prevent rainwater accumulation. During each session in the first year of the study, we set up 49 traps, at a distance of five m from each other, on both the ecotone and the more open grass areas. However, only one individual was found in a trap, suggesting this method is unsuitable for the species.

The Snake-eyed Skink generations overlap over time due to the delayed maturation of some individuals. This species is generally sedentary, likely without performing many long-distance movements more than 60 m and usually maintaining a small individual range. Uneven recapture by seasons was due to the secretive lifestyle of the species and the overgrowing of grasses and/or bushes of the habitat, with the change of seasons decreasing recapture rates. In future population studies on species with biology similar to that of *A. kitaibelii*, we stress the importance of using a method that will provide an increased recapture rate and implementing the Robust Design (Kendall & Bjorkland 2001).

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Appendix 1. Average distances between (re)capture-recapture of *Ablepharus kitaibelii,* by season. Included are all recaptures, including within the same seasons. *n* = number of recaptures. (1) denotes a season one calendar year after the previous (re)capture, (2) – two years after, (3) – three years after.

	n	Distance (m)	Min.	Max.	SD
Summer – Autumn	45	7.86	0.39	34.37	6.84
Autumn – Autumn	37	8.24	0.96	52.05	11.88
Spring – Spring	30	6.11	0.49	20.60	4.26
Autumn – Spring (1)	28	8.68	0.89	53.26	10.52
Spring – Summer	22	8.52	0.92	29.26	7.52
Summer – Summer	18	7.55	1.83	23.68	5.83
Spring – Autumn	16	8.92	1.38	31.00	8.91
Summer – Spring (1)	10	12.88	2.84	39.10	11.21
Spring – Spring (1)	8	7.11	3.09	16.29	4.61
Autumn – Summer (1)	7	20.11	4.47	55.49	17.57
Autumn – Autumn (1)	5	5.96	1.45	18.92	7.28
Spring – Summer (1)	3	15.02	4.02	34.02	16.52
Summer – Summer (1)	3	12.33	8.45	19.85	6.51
Summer – Autumn (1)	3	7.41	3.05	10.91	4.00
Autumn – Spring (2)	2	22.18	5.32	39.03	
Autumn – Summer (2)	2	23.20	14.69	31.72	
Spring – Spring (3)	1	6.36			
Spring – Autumn (1)	1	36.32			
Summer – Spring (2)	1	3.32			
Total	242	8.88	0.39	55.49	9.44