



Persistent Winter Activity in *Triturus ivanbureschi* Arntzen & Wielstra, 2013 (Amphibia: Caudata)

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Abstract: Most amphibians from the temperate climate zones hibernate during the winter, and although incidental observations of winter activity are not rare, systematic data are still lacking. The study was conducted throughout the astronomical winter (December 2020 – March 2021) in a pond near Sofia, Bulgaria. Funnel traps were set each week for the duration of the study and the ventral side of all captured newts was photographed to allow for individual identification. The following parameters were measured: dissolved oxygen in the water, air and water temperature. Results demonstrated that both male and female newts were active in the water throughout the winter period, even when the surface of the pond was frozen solid (at times, the ice was thick enough for a human to safely walk on the pond). As early as the beginning of January, males had very prominently developed crests. A total of 153 individual newts were captured, with 79 (51.63 %) of them recaptured at least once. Recaptures were used to calculate the maximum active population size, which was 142 ± 9 (125–159) for males and 94 ± 13 (69–118) for females. The number of captured newts each session correlated very closely with estimated active population size but there was no apparent correlation with the values of the measured parameters.

Key words: Buresch's crested newt, oxygen, population dynamics, temperature.

Introduction

As poikilothermic animals, amphibians are especially sensitive to changes in ambient temperature, and most species from the temperate climate zones hibernate during the colder months of the winter. In recent decades, climate change has affected this behaviour and rising temperatures have caused many anurans and urodeles to emerge from hibernation earlier than in the past (GRIFFITHS et al. 2010, GE et al. 2015). This phenomenon has been well-documented in explosively-breeding anurans (TRYJANOWSKI et al. 2003, NEVEU 2009) but newts, with their secretive lifestyle, present a harder challenge. Winter hibernation in newts is mostly on land, i.e. under rocks, tree trunks and rodent tunnels; however, aquatic overwintering

is regularly observed in deeper waters (FAHRBACH & GERLACH 2018). While incidental observation of late-autumn and winter activity have been reported for a number of newt species (CHADWICK et al. 2006, KACZMAREK et al. 2018), the data are incomplete or scattered in the literature.

Our study aims to provide a detailed description of the activity pattern of the Buresch's crested newt *Triturus ivanbureschi* Arntzen & Wielstra, 2013 for the entire winter season in a region with a temperate climate.

Materials and Methods

The Buresch's crested newt is distributed from the south-eastern parts of the Balkan Peninsula to the

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western parts of Anatolia (WIELSTRA et al. 2013, 2018). Bulgaria is at the north-western limits of the species range, where it occurs in the central and southern parts of the country between 0–1700 m a. s. l. but is absent along the Danube River (STOJANOV et al. 2011).

The study pond is located near the city of Sofia, Bulgaria (42.595N, 23.368E, 858 m a. s. l.). It has an approximate surface area of around 150 square meters and is sustained by snowmelt and rainfall; its location within a depression in the terrain allows it to contain water for prolonged periods, sometimes all-year round. The study was conducted throughout the astronomical winter (21 December 2020 – 20 March 2021) and each week funnel traps were set in the late afternoon and checked in the following morning. For approximately half of the duration of the study, the surface of the pond was frozen solid, so in order to set and collect the traps, a pickaxe was used to break the ice. The following parameters were measured at the set-up and collection of the traps: dissolved oxygen in the water (measured in mg/l with a DO Meter Tester model 8403, accuracy of +/- 1.5 % and resolution of 0.01), water and air temperature (measured in °C with a digital thermometer, accuracy of +/- 0.1 °C). The ventral side of all captured newts was photographed to allow for individual identification, after which they were released back into the pond. Identification was facilitated by using the image recognition software Hotspotter ver. 1.0 (CRALL et al. 2013), with potential matches always manually verified by the authors (for the full procedure, see NAUMOV & LUKANOV 2018). This mark recapture technique allowed us to estimate the active population size during the study period using the program MARK v.

9.0 (WHITE & BURNHAM 1999). We chose the Jolly-Seber model for open population as formulated by SCHWARZ & ARNASON (1996) because it would account for the probability of a newt entering the pond during the study. Model fit was determined using the lowest value for the Akaike's Information Criterion (AIC) and model notations were as follows: survival rate (Φ), capture probability (p), probability of entrance (b) and population size (N). Model results are presented as Mean \pm Standard error (Minimum–Maximum). All data were tested for normality using the Shapiro-Wilk test and the null hypothesis was rejected. Population estimates were tested for correlation with the measured parameters and the number of captured newts per session using a Spearman rank order test in Statistica 10.0 (STATSOFT, INC. 2011), with level of significance set at $p < 0.05$. For all parameters, a mean value (i.e., the average from the two measurements made at the set-up and collection of the traps) was used in the analysis. As the water depth varied greatly (~30 cm in December to ~100 cm in February and March) the number of funnel traps gradually increased in order to cover roughly the same portion of the pond area throughout the study, and the number of captured newts per trap was also taken into consideration for the analysis.

Results

A total of 153 individual newts were captured, with a sex ratio of almost exactly 2:1 (103 males and 50 females) (Table 1). The overall recapture rate was 51.63 %, with 62 males and 17 females recaptured and identified at least once; the recapture rate

Table 1. Measured parameters presented as Mean (Afternoon–Morning value) and captured newts for each of the 11 weekly sessions. Abbreviations: DO, dissolved oxygen; WT, water temperature; AT, air temperature; Cs, number of captured newts; Cs/t, number of captured newts per trap.

Month	DO (mg/l)	WT (°C)	AT (°C)	Cs	Cs/t
December	9.0 (12.6–5.4)	6.6 (6.9–6.2)	7.8 (7.5–8.1)	11 (8♂, 3♀)	0.9 (0.6♂, 0.3♀)
January	10.0 (13.0–7.0)	6.2 (7.5–4.8)	8.3 (10–6.5)	11 (8♂, 3♀)	0.9 (0.6♂, 0.3♀)
January	11.5 (13.2–9.8)	2.2 (2.5–1.8)	-0.6 (1.8– -3)	23 (14♂, 9♀)	1.9 (1.1♂, 0.8♀)
January	12.9 (13.9–11.9)	1.6 (2.5–0.7)	4.8 (8.5–1)	14 (12♂, 2♀)	1.2 (1♂, 0.2♀)
January	7.9 (8.2–7.5)	1.9 (3.4–0.5)	7.5 (3–12)	8 (5♂, 3♀)	0.6 (0.4♂, 0.2♀)
February	5.2 (4.7–5.7)	5.5 (5–6)	14.5 (16.4–12.5)	43 (34♂, 9♀)	2.2 (1.7♂, 0.5♀)
February	8.3 (8.8–7.8)	2.5 (3.5–1.5)	-3.8 (-7.5–0)	25 (16♂, 9♀)	1.7 (1.1♂, 0.6♀)
February	11.4 (11.7–11.2)	2.9 (2.2–3.5)	6.0 (4–8)	28 (15♂, 13♀)	1.5 (0.7♂, 0.8♀)
March	10.45 (10.4–10.5)	3.9 (5.3–2.5)	8.8 (10.4–7.1)	30 (25♂, 5♀)	1.5 (1.2♂, 0.3♀)
March	13.1 (13.5–12.7)	5.3 (5.5–5.1)	11.1 (10.5–11.7)	53 (43♂, 10♀)	2.1 (1.7♂, 0.4♀)
March	13.2 (12.8–13.5)	5.0 (5.2–4.8)	2.3 (3.3–1.3)	48 (40♂, 8♀)	1.9 (1.6♂, 0.3♀)

was highest in March, reaching 70.83 % in the last session (for details, see Supplementary files). The three models in which survival rate, capture probability and probability of entrance depended on the group (male/female) carried 99 % of the cumulative model weight and had the lowest and very similar AIC values (829.95–830.96). They only differed for the estimate of population size, which was constant, or depended on time or group. For this reason, model averaging was performed. Survival rate for both males and females was $\Phi=1.00$ with virtually no variation, which is expected, considering the short period of the study. Capture probability was $p=0.27\pm 0.024$ (0.22–0.32) and $p=0.13\pm 0.021$ (0.09–0.18) for males and females, respectively. The probability of entrance was $b=0.08\pm 0.001$ (0.07–0.09) for males and $b=0.07\pm 0.001$ (0.05–0.10) for females. The overall active population size at the end of the study was estimated to be 142 ± 9 (125–159) for males and 94 ± 13 (69–118) for females. Although the population estimates corre-

lated strongly with the number of captured newts ($R=0.83$, $p<0.01$), and almost significantly with the captured newts per trap ($R=0.58$, $p=0.06$), neither of them was correlated to the measured parameters. For population estimates, results were as follows: DO – $R=0.42$, $p=0.20$; WT – $R=-0.08$, $p=0.81$; AT – $R=0.05$, $p=0.87$, and for the number of captured newts: DO – $R=0.44$, $p=0.17$; WT – $R=0.21$, $p=0.52$; AT – $R=0.23$, $p=0.49$.

In addition to the Buresch's crested newts, we also caught a total of 46 smooth newts *Lisso-triton vulgaris* (40 males, 6 females) and 46 adult agile frogs *Rana dalmatina*. While significant, these numbers were still not sufficient for realistic active population estimates, so we did not include them in our analysis. Tellingly, agile frogs were not caught from mid-January to mid-February, and only three male smooth newts were caught during the same period, thus strongly indicating that these species cease their activity during the coldest periods.



Fig. 1. A male newt with prominent crest (a) and an overall view of the study pond (b) from the second week of January.

Discussion

The observed sex ratio could corroborate the suggestion of JEHLÉ et al. (2011) that males over-winter in ponds in larger numbers than females; however, it has to be noted that the sex ratio was even more male-biased in March. The estimated active population size is in accordance with the accepted typical size for crested newt populations, which is around 200 adults (JEHLÉ et al. 2011). Regarding the measured parameters, the lack of effect of dissolved oxygen levels could be expected in view of the results of LUKANOV et al. (2021) who also found no correlation between DO levels and captured newts. Nevertheless, the latter study established a negative correlation between the number of captured crested newts and both air and water temperatures. However, that study was conducted from early spring to late autumn and included the much higher summer temperatures, when water level dropped drastically and most newts left the ponds. The present results could indicate that low temperatures are of no great importance for this species as long as they are close to the average values for the period (i.e., not extreme). This suggestion is further corroborated by the fact that males had very prominent crests as early as the first half of January when the pond was already frozen solid (Fig. 1).

Gravid females were also caught in this period, similarly to the published observations for other species of the genus (CVETKOVIĆ et al. 1996, FAHRBACH & GERLACH 2018). In our case, ice covered the pond from the second week of January to the beginning of March, but partially and temporarily melted during a few particularly warm days in the first half of February. This partial melting increased the pond area and depth, which probably triggered more newt activity, as even during the subsequent cold period, when the pond froze solid again, we still caught more animals than in the previous sessions (Table 1). Unfortunately, water area and depth could not be precisely measured, and we could not quantitatively analyse the effect of melting ice and snow. Regarding the air temperature, it has to be noted that for the region of Sofia, all of December (+3.7 °C), January (+1.8 °C) and February (+3.3 °C) had higher average temperature in comparison to a reference period of 1981-2010 (STRINGMETEO 2006). March, however, had lower values (-2.3 °C) and was the month with most captured newts, again indicating the apparently insignificant effect of temperature during this period and confirming the observation of JEHLÉ et al. (2011) that most crested newts arrive at their ponds in the second half of March. Regarding migration to the

pond, it has to be noted that while incidental over-winter activity has been observed in *T. cristatus* (see FAHRBACH & GERLACH 2018), the first migrants are usually recorded when night air temperatures remain above 4-5 °C (JEHLÉ et al. 2011). Although we could only record afternoon and morning temperatures, even from them it is evident that night temperatures could not have been above 5 °C for the larger part of the study period. This leaves two possibilities for *T. ivanbureschi*: 1) most newts arrived at the pond with the February warming (possible, considering the onset of migration occurs within 10 days and mass migrations can be observed after a long phase of bad weather conditions followed by mild rains (JEHLÉ et al. 2011) although night temperatures were still below 5 °C); and 2) newts actively migrated in lower temperatures. The second possibility is supported by the study of HARPER et al. (2019), who have found active *T. cristatus* on the ground in air temperatures as low as 0.7 °C (although they still caught most newts in the 4–5 °C range, and water temperature was 7.5–10.9 °C). What is certain is that there were active newts underwater during the whole winter (incl. recaptures from December, January, February and March – see Supplementary files) – which questions the supposedly short aquatic phase for this species, set at 3 months by IVANOVIĆ et al. (2012) (in their study referred to as *Triturus karelinii* sensu lato). In the pond of the present study, we have observed newts in the water for each month of the year, highlighting the need for additional studies of this species life cycle. Considering the low probability of entrance and the relatively high recapture rate for the period of the study, we hypothesize that most newts remain in the water for as long as the water level and the upper temperature limits would allow. The decline of the number of captured newts (both absolute and as captures per trap) from mid-January to the beginning of February and the falling oxygen levels at that time (Table 1) could indicate that without the warmer days in February, newt activity would have subsided, or the active newts would have drowned under the ice. If the presently reported aquatic overwintering is typical of the species, it could be a major limiting factor for its distribution further north. However, it should also be noted that during these weeks we had the most problems breaking the ice, so positioning of the traps was not optimal (i.e., they were more clustered together and covered less area).

While KACZMAREK et al. (2018) observed terrestrial activity for *L. vulgaris* in January, the reported air temperatures of 11 °C and 9 °C were higher than the average in our study. A single male *L.*

vulgaris, active on land in temperatures just above freezing, was reported by JABLONSKI (2013), suggesting a lower temperature threshold for this species. It is possible that populations of *L. vulgaris* and *R. dalmatina* in the pond were much smaller than that of the Buresch's crested newts and the percentage that remained active were just too few to detect.

In conclusion, it has to be said that while overwintering in water is to be expected for all representatives of the *Triturus* species group, this study reports the first systematic data on underwater winter activity for a poorly studied member of the genus. Both males and females were active in low temperatures, under solid ice, and were in breeding condition in mid-winter. These results suggest that the species is more tolerant to low temperatures than previously thought. Further research should be focused on the duration of its aquatic phase.

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Data accessibility: Mark-recapture data are available as a supplementary file in MS Excel format (http://www.acta-zoologica-bulgarica.eu/2021/002573_suppl.xlsx). Any other data associated with the study are available upon request from the corresponding author.

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