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Effects of selected environmental parameters on the activity and body condition of the Buresch's crested newt (*Triturus ivanbureschi*) with notes on skin secretions

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Abstract. As amphibians with pronounced biphasic lifestyle, crested newts are dependent on available ponds throughout their breeding season, but data on parameters that influence their abundance and activity is largely lacking. In the present study, we tested the possible effects of five parameters (air and water temperature, dissolved oxygen levels, acidity and oxidative reduction potential) on newt populations from adjacent ponds across two years. Although pond size was different (small vs large), both ponds were temporary, and funnel traps were set each week from the beginning of March until the drying of the ponds. Parameters were measured twice per week (i.e., in the evening and in the following morning) and were checked against the total number of captured newts. A portion of the captured newts was measured and weighted in order to compare newts from both ponds by body condition index (BCI). In addition, cotton swabs were used to collect skin secretion from some of the captured newts, which were tested for the presence of alkaloids. Expectedly, temperature had a pronounced effect on newt activity in both ponds, but levels of dissolved oxygen and oxidative reduction potential had no effect. Acidity was only relevant when higher daily amplitudes were registered, and in that case, it had a negative effect on newt activity. Overall, the large pond presented more stable conditions with male and female newts having a higher BCI compared to those from the small ponds. Although no alkaloids were found in the skin secretions, some of their possible precursors (sterol compounds) were detected.

Key words: aquatic phase, activity, Salamandridae, habitat quality.

Introduction

Habitat selection and spatial distribution of organisms are directly linked to habitat quality, and understanding the exact relationships affecting these processes is of key importance in establishing patterns in species distribution and abundance (Boyce et al. 2016). Amphibians are organisms with complex life cycles that require both terrestrial and aquatic habitats (Wilbur 1980), making the study of their habitat choices particularly important (Winandy et al. 2017). Traditionally, members of the Salamandridae family are accepted to show a strong breeding site fidelity, combined with low vagility, which is typical for metapopulation structures (Smith & Green 2005). However, many environmental conditions could be highly unpredictable, or vary greatly on a short-term temporal basis, putting such species at risk – for this reason, a better understanding of the environmental conditions with direct relation to species habitat preferences is required.

Crested newts (*Triturus cristatus* superspecies) are typical representatives of the Salamandridae family with a biphasic lifestyle (aquatic and terrestrial phase), and they usually migrate within a few hundred metres of their home pond (Denöel et al. 2013). At the same time, some studies have suggested a more active habitat selection within this limited radius, with newts avoiding aquatic patches with unfavourable conditions (Resetarits 2005). While predation risk and resource availability have been established as among the main driving factors behind this habitat selection (Amburgey et al. 2014, Idermaur et al. 2010), there is still a significant lack of data on the effect of various abiotic conditions, especially in temporary pond that are characterized by high variability. Pond surface area and depth, as well as type and abundance of underwater vegetation are also known to be important factors (Idermaur et al. 2010), but again display great variability in small temporary ponds.

In view of the above, it is important to assess the physiological state of individual newts, as this is indicative not only of foraging success, but also of ability to cope with environmental pressure (Jakob & Marshall 1996). Variations in environmental conditions can have a cascading effect on the growth rate and fitness of individuals (Lunghi et al. 2018), and Body Condition Index (BCI) estimates have been widely used in a large number of studies on animal populations, incl. newts (e.g., Cooke & Arnold 2003, Kopecký et al. 2010, Jarvis 2015).

Amphibians are well known for their skin secretions – however, environmental stressors have been demonstrated to negatively affect chemical defense capabilities (Robak et al. 2019). Alkaloids that have been detected in amphibian skin, in particular, are represented in over 20 structural classes (Daly et al. 2005). While chemical composition of skin secretions in the Fire Salamander (*Salamandra salamandra*) – another salamandrid species – is well studied and documented (see Knepper et al. 2019), data on newts is largely limited to few short communications (e.g., Yotsu-Yamashita et al. 2007).

In the present study, we investigated two newt populations inhabiting temporary ponds with different ecological conditions. Selected environmental parameters were measured in an effort to establish their relation to newt abundance and body condition. We hypothesised that variations in parameters would have negative effect on newts, reducing their abundance, body condition and defense capabilities.

Material and Methods

The Buresch's crested newt *Triturus ivanbureschi* Arntzen & Wielstra in Wielstra, Litvinchuk, Naumov, Tzankov, & Arntzen, 2013, is part of the crested newt species complex and is distributed in Southeastern Europe and parts of Anatolia (Wielstra et al. 2013, Wielstra & Arntzen 2016). In Bulgaria it occurs across most of the country at elevations between 0 and 1700 m above sea level, but it is absent along the Danube river and the lower currents of its tributaries, where *Triturus dobrogicus* (Kiritzescu, 1903) is present (Stojanov et al. 2011, Wielstra et al. 2017).

The studied populations inhabit two ponds ("small" - 6m in diameter, and "large" - 27m in diameter) north of the village of Bistritsa (42.595°N, 23.368°E) at around 850m above sea level, near the city of Sofia. Distance between ponds in a straight line is around 200m, with landscape composition in the area consisting mainly of wild grasslands with dense patches of shrubs and small trees. Both ponds are temporary – filling with water during snowmelt in early spring and drying up in late summer or the beginning of autumn. In late spring and summer, the large pond is overgrown with bulrush (*Typha* sp.), yellow iris (*Iris pseudacorus*) and common bladderwort (*Utricularia vulgaris*), while only the latter is present in the small pond. In 2018 and 2019, funnel traps (as described in Griffiths & Inns (1998), mesh diameter 3 mm) were set each week from the beginning of March until the drying of the ponds (mid October in 2018 and late August in 2019). Traps were set up in the evening and collected in the following morning. For each pond, trap exposure time was calculated as the difference between the hour of set-up and the hour of collection of the traps. This was multiplied by the number of traps (average 7 for the small pond and 22 for the large pond, depending on remaining water) and the resulting value was used as a denominator, with total number of captured newts used as a numerator – resulting in the relative ratio of captured newts per trap-hour, which was used in subsequent analyses. Using publicly available astronomical calendars for 2018 and 2019 for Bulgaria, we also factored day duration (time from sunrise to sunset) on the dates when traps were set.

We measured the following environmental parameters in order to test their effect on newt activity: dissolved oxygen (DO) in the water (measured in mg/l with a DO Meter Tester model 8403), water and air temperature (WT and AT, respectively; measured in ºC with a digital thermometer), acidity (pH) and oxidative reduction potential (ORP) (both measured with a Multipurpose Pen type Meter model M0199720). All parameters were measured before the set up and the collection of the funnel traps (i.e., twice each week during the active season, in the evening and in the following morning).

On six occasions during breeding season in 2019, a total of 180 individual newts (121 males and 59 females) were measured (snout–vent length (SVL) was taken with a ruler, accuracy of 0.1cm) and weighted (body mass taken with a Durascale digital pocket scale, accuracy of 0.01g). Body condition index (BCI) was calculated as the ratio of log (body mass) on log (SVL). All newts were released at the sites of capture. Individual recognition was achieved by comparing photographs of the ventral coloration pattern of recaptures.

Skin secretions from *T. ivanbureschi* were collected during breeding season in 2019, and analysed in 2020. Handling of captured newts caused them to excrete fluids that were absorbed by gently scrubbing them with a cotton swab from the snout back towards the tail, similar to the non-invasive method used for fire salamanders by Knepper et al. (2019). The cotton swabs from a total of 95 specimens from the large pond (85 males, 10 females) were stored in a refrigerated sterile flask and extracted three times with ethanol. Because of the low number of females, all swabs were combined, evaporated under reduced pressure to dryness (18.5 mg) and processed in order to obtain the alkaloid extract (3.2 mg) (Doncheva et al 2014). The obtained extract was further analysed by gas chromatography mass spectrometry (GC-MS), with all measurements following the methodology described in Doncheva et al. 2014.

All data was tested for normality with the Shapiro-Wilk test and the null hypothesis was rejected for all parameters except BCI. A Mann-Whitney U test was used to test for differences in the measured parameters between the two ponds and across both years; this test was also used to test for differences in SVL and body mass between newts of both sexes. A t-test and linear regression were performed to compare BCI between males and females from the two ponds. A Spearman rank order correlation was used to test relations between SVL and body mass, as well as between the number of captured newts, day duration and the measured environmental parameters. All tests were performed with the computer program Statistica v.7.0 (StatSoft, Inc., 2004), and the chosen level for statistical significance was $p<0.05$.

Results

In both ponds, the overall number of total captured newts differed across years – for the small pond, it was higher for 2018 compared to 2019, while for the large pond the opposite was true (Fig. 1). Overall, day duration had a small, but significant negative correlation to the number of captured adult newts (Spearman rank order correlation $R=-0.324$, $p<0.001$).

A descriptive summary of the measured environmental parameters for both ponds is presented in Table 1. A Mann-Whitney U test revealed there were no statistically significant differences in the parameters between 2018 and 2019, so they were combined for subsequent analyses (AT $U=5245.000$, $p=0.105$; WT $U=5823.000$, $p=0.679$; DO $U=4959.000$, $p=0.262$; pH $U=251.000$, $p=0.312$; ORP $U=349.000$, $p=0.403$). According to the Mann-Whitney U test, there were statistically significant differences between ponds in four out of the five parameters: WT ($U=5305.00$, $p=0.028$), DO ($U=1570.50$, $p<0.001$), pH ($U=191.500$, $p<0.001$) and ORP ($U=439.000$, $p<0.001$), with AT the only exception ($U=6122.000$, $p=0.599$). Overall values for the small pond were higher than those for the large pond (Table 1).

It has to be noted that while in the small pond all parameters also varied according to time of measurement (i.e., evening/morning), in the large pond pH and ORP were rather stable (Table 2). Expectedly, the Spearman rank order correlation revealed a significant negative correlation between both mean air and mean water temperature and the number of captured newts for both ponds; results for the other measured parameters were mixed (Table 2). Amplitude (difference between parameter values measured in the evening/morning) was not correlated with the number of captured newts with one notable exception – pH for the small pond (mean overnight amplitude of 0.354 (-0.100-1.170 ± 0.303); Spearman rank order correlation: females $R=-0.438$, $p=0.005$; males $R=-0.396$, $p=0.013$; total adults $R=-0.394$, $p=0.013$).

As could be expected, there was a significant positive correlation between SVL and body mass (Spearman rank order correlation $R=0.704$, $p=0.002$), with females being generally larger and heavier than males (Mann-Whittney U test, SVL $U=2671.50$, $p<0.001$; body mass $U=2485.50$, $p<0.001$). Comparison between BCI of males and females from both ponds revealed statistically significant differences in all cases, with higher overall values for newts from the large pond (t-test, females ($n=57$) $t=4.245$, $p<0.001$; males ($n=119$) $t=2.243$, $p=0.027$) (Table 3, Fig. 2).

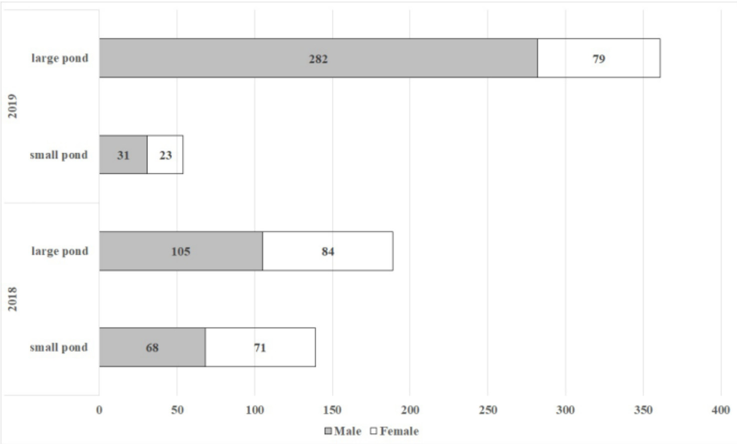

Figure 1. Total number of adult newts captured during the study period.

Table 1. Values for the measured environmental parameters. Data is presented as median, minimum and maximum values and lower (25%) and upper (75%) quartile. Statistically significant differences between ponds are marked with an asterisk. For abbreviations, see Materials and methods.

	Small pond			Large pond		
	Median	Min-Max	25-75%	Median	Min-Max	25-75%
AT	20.625	-1.500-27.500	17.000-23.250	21.000	-1.000-29.500	16.000-23.500
WT*	19.275	4.500-26.500	15.400-22.500	17.500	3.100-25.000	12.250-20.750
DO*	9.363	1.585-18.000	7.200-12.250	2.575	0.700-11.250	1.475-4.450
pH*	7.825	7.300-8.290	7.500-8.015	7.050	6.850-7.45	6.955-7.255
ORP*	129.000	71.000-179.500	108.500-145.000	84.000	48.500-170.500	7.750-110.500

Table 2. Results of Mann-Whitney U test on variation in parameters according to time of measurement (evening/morning) (first results row for each pond) and Spearman rank order correlations between mean values for the parameters and the number of captured newts. Statistically significant results are marked with an asterisk. For abbreviations, see Materials and methods.

		AT	WT	DO	pH	ORP
Small pond	Daily variation	U=705.500, *p<0.001	U=719.000, *p<0.001	U=397.000, *p<0.001	U=409.000, *p<0.001	U=58.000, *p<0.001
	Females	R=-0.480, *p<0.001	R=-0.559*, *p<0.001	R=0.315, *p=0.016	R=-0.131, p=0.427	R=0.055, p=0.821
	Males	R=-0.424, *p<0.001	R=-0.504,*p<0.001	R=0.151, p=0.258	R=-0.179, p=0.276	R=0.482, p=0.099
	Total adults	R=-0.509, *p<0.001	R=-0.592, *p<0.001	R=0.251, p=0.058	R=-0.138, p=0.403	R=0.302, p=0.208
Large pond	Daily variation	U=643.000, *p<0.001	U=850.000, *p<0.001	U=838.000, *p<0.001	U=388.500, p=0.197	U=235.500, p=0.282
	Females	R=-0.136, p=0.320	R=-0.166, p= 0.227	R=0.046, p=0.738	R=-0.010, p=0.783	R=-0.151, p=0.482
	Males	R=-0.533, *p<0.001	R=-0.605, *p<0.001	R=0.625, *p<0.001	R=0.442, *p=0.013	R=0.499, *p=0.013
	Total adults	R=-0.475, *p<0.001	R=-0.539, *p<0.001	R=0.485, *p<0.001	R=0.320, p=0.079	R=0.382, p=0.065

Table 3. Values for the measured SVL, body mass and BCI, presented as Mean ± SD (Min-Max). SD – standard deviation.

		SVL (cm)	Body mass (g)	BCI
Small pond	Males, n=16	7.563 ± 0.376 (6.800-8.100)	11.506 ± 1.955 (8.800-15.260)	1.201 ± 0.063 (1.087-1.318)
	Females, n=31	7.997 ± 0.451 (6.800-8.900)	13.475 ± 2.771 (8.200-21.970)	1.242 ± 0.082 (1.037-1.444)
Large pond	Males, n=105	7.870 ± 0.456 (6.800-8.700)	13.324 ± 2.589 (7.750-20.350)	1.246 ± 0.076 (1.023-1.393)
	Females, n=28	8.168 ± 0.678 (6.900-9.800)	16.472 ± 3.338 (9.770- 24.880)	1.325 ± 0.067 (1.180-1.433)

The low volume of the concentrated skin secretions extract (3.2 mg) did not allow for the initially planned test for correlation between parameters and skin secretions. The GC-MS analyses revealed no alkaloids present, but a sterol compound (cholest-5-en-3-ol) was reliably detected.

Discussion

Overall, the present study demonstrates that even habitats of the same type (i.e., temporary ponds) offer rather different conditions in terms of environmental parameters that could affect newt populations. The measured environmental parameters did not vary across years, however, the number of captured newts did – this could be attributed to the more general climatic conditions for the respective years, as 2018 had much higher levels of rainfall than 2019 (for the area of Sofia – 124% vs. 75% of the standardized norm, data by the Executive Environmental Agency of Bulgaria). More rain would mean that both temporary ponds could contain water for longer periods, which is especially beneficial for the small pond, as it dries up more quickly.

Air temperature was the only parameter that did not vary between ponds, which is expected considering the

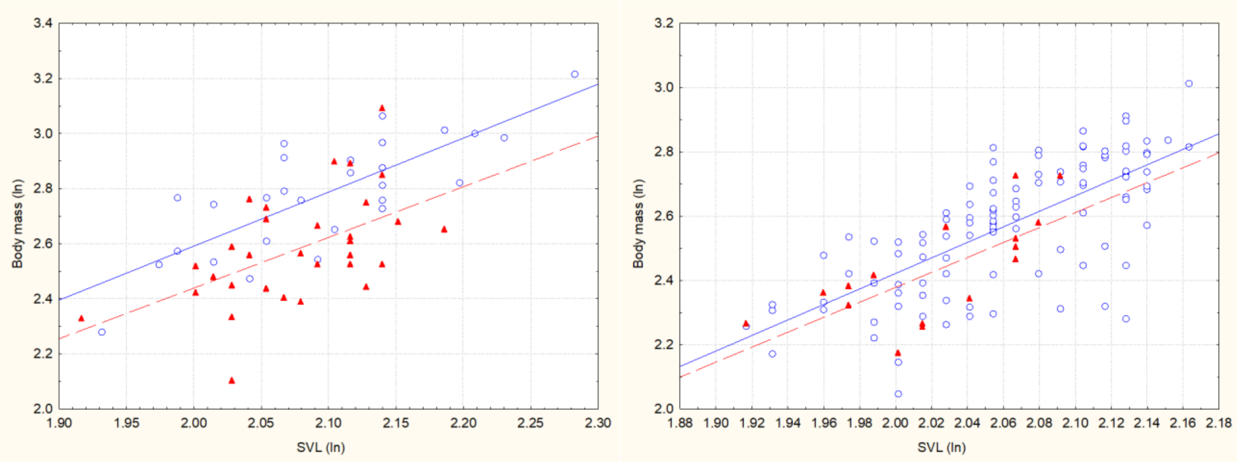

Figure 2. Linear regression of BCI for females (left) and males (right) from the small pond (dashed line, full triangles) and the large pond (solid line, open circles). See Table 3 for the exact values.

distance between them and their similarity in terms of exposition and altitude. This is also reflected by the similar vegetation around and within the two ponds, although more aquatic plant species were registered in the large pond. Results related to temperature and day duration could easily be explained by the specifics of the biphasic lifestyle – as days become longer and temperatures rise, the aquatic phase draws to a close and the terrestrial phase begins, resulting in an increasingly lower number of captured newts. Dependency between mean temperature values and newt phases has been well-documented in other closely related species (Baker et al. 2011), so it is no surprise that the Buresch's crested newt follows the pattern established for other members of its group.

In regards to the other measured parameters, to our knowledge this is the first attempt to establish their role for newt populations. There is some data on acidity in natural ponds as abiotic factor for breeding site selection in anurans (Indermaur et al. 2010), and in terms of mean values our results are very similar to those of the cited study: 7.800 ± 0.311 [6.940-8.528) (Mean ± SD (Min-Max)]. However, pH in that study was measured 4 times per month, while our method of measurement (evening/morning) allowed us to effectively double this number and check for overnight variations. Such variations were only registered in the small pond and they had a noticeable negative effect on the number of captured newts. Dolmen et al. (2017) demonstrated that several amphibian species, incl. newts, were able to recover their populations from strong acidifications of their home ponds after pH levels gradually rose throughout the next two decades. Interestingly, the authors list an average pH increase of just 0.14 per year, while the mean overnight amplitude in the small pond was 0.354 (although pH values did not differ across years). It is possible that, given acidity values are within the habitable thresholds (above 5.3 for other Crested newts, see Dolmen et al. 2008), the scale of temporal variation, rather than mean value, is more important for newt abundance.

While it is known that dissolved oxygen affects the development of amphibian larvae (Wassersug & Seibert 1975), its role in adult newts is yet to be clarified. Our results give reason to suggest that oxygen concentration is of limited importance for adult newts – no correlation between number of newts and DO levels was established in the small pond, and in the large pond it was only present for males and total adults. It could be hypothesised that oxygen levels only affect newts in larger ponds after the breeding season, when abundant underwater vegetation could, to some extent, counter the effects of increased water temperature.

Most difficult to interpret are correlations between mean pH and ORP values and number of captured newts. These were not significant in the small pond, but in some cases appeared significant in the large pond; however, pH and ORP values did not actually vary in the large pond, as indicated by the Mann-Whitney U test (see Results). Considering that in all cases p values were near 0.05, conclusions can be defined only after more measurements, preferably in controlled conditions.

Body condition is positively related to fat contents, making BCI a reliable estimate for storage of energy reserves (Denöel et al. 2002). While BCI in amphibians can vary over time (Băncillă et al. 2010) and across seasons (Jarvis 2015), our results suggest that it can also vary between ponds and can be used as a proxy for habitat suitability. Differences between sexes can be due to sex-specific allocation of resources and fat reserves (Jarvis 2015), as females are generally larger than males, with sexual dimorphism index of 0.089 (Lukanov & Tzankov 2016). Differences between ponds are more likely to be due to the more stable conditions offered by the larger pond (e.g., contains water for longer periods of time, contains more vegetation, potentially more prey items, less variation in measured parameters).

Regarding alkaloid presence and variability in newt skin secretions, our results are inconclusive. Sterol compounds have been suggested to serve as precursors in the biosynthetic pathway of amphibian alkaloids (Habermehl & Haaf 1968). However, results from the few existing studies are inconclusive and more experimental evidence is needed (Mebs & Pogoda 2005). Considering that Yotsu-Yamashita et al. (2007) sacrificed 59 *Triturus* newts and detected tetrodotoxin in only 15 of them, the contents of newt skin secretions is a topic in need of further development.

In conclusion, it could be summarized that comparison between the two ponds highlights the effects of temperature and acidity variations, while oxygen-related parameters appeared to be of lesser importance. Understanding variations in habitat conditions on a local scale is important not only in terms of scientific knowledge, but also for conservation, as most newt species are protected by national and European law.

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