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First record of underwater sound produced by the Balkan crested newt (*Triturus ivanbureschi*)

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Abstract. This study presents first evidence for underwater sounds produced by adult Balkan crested newts (*Triturus ivanbureschi*). Recordings were made in spring of 2019 in controlled laboratory conditions using a commercially available omnidirectional hydrophone connected to a linear PCM recorder. A total of 27 animals (21 males, 6 females) were recorded under different conditions: (a) alone in an empty tank, (b) alone in a tank full of vegetation, and (c) a pair in an empty tank. Results indicated that both male and female newts produced a click-like sound with a mean duration of 34 ms (± 5.31 SD; range: 27-51) and mean peak frequency of 1887 Hz (± 405 SD; range: 1162-2770). Not all newts tested produced sounds and there were no statistically significant differences between males and females or recordings under different conditions in terms of click number, duration and frequency parameters, with the exception of the ratio of peak frequency/bandwidth at 50% peak amplitude, which was lower for clicks produced in the vegetated tank. Newt snout-vent length and body mass also had no effect on any of the studied parameters. The obtained results suggest that clicks could have a function in orientation and exploratory behaviour.

Keywords. Clicks, conditions, environment, interaction, orientation, phase.

INTRODUCTION

Sound production plays an important role for many taxa across the animal kingdom. For amphibians, this is mainly true for anurans – the first early attempts at more detailed studies of the functions of vocal signals in anurans date from the first half of the 20th century (Noble and Noble, 1923), and the first comprehensive work on acoustic communication in amphibians and reptiles is by Bogert (1960), which is also the first attempt to view the information of amphibian vocal signals in an evolutionary and ecological frame. In recent decades there is a growing number of publications describing the structure of anuran calls (e.g., Ziegler et al., 2011; Guerra et al, 2018; Stanescu et al., 2018; Carvalho et al., 2019), geographic variations of vocal signals in some species (e.g., Amezquita et al., 2009; Kaefer and Lima, 2012), as well as the role of the signals as phylogeographic indicators (e.g., Stöck et al., 2008; Vences et al., 2013).

In contrast, caudate amphibians have received very little attention in this regard. While they lack a tympanic middle ear, a number of studies have established that their underwater auditory abilities are sufficient for them to sense sound frequencies by other means, such as their mouths or lungs (e.g., Hetherington and Lombard, 1983; Christensen et al., 2015). Available data on their sound production is limited to just a few taxa, and even then, the information is mostly descriptive, with little effort to analyse its ecological function. In addition, most of the data is on North American species, leaving the rest of the world almost unstudied. Even though a variety of sounds made by caudate amphibians have been described as early as the mid-twentieth century (Maslin, 1950; Neil, 1952), they were assumed to be mostly non-func-

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tional and produced by unintentional expulsion of air (Bogert, 1960). On land, some caudate species are known to produce squeaking noises when stressed ("mouse-like squeaking note"; Maslin, 1950). Although a number of authors have registered underwater hisses, clicks or squeaks in various species (Maslin, 1950; Gehlbach and Walker, 1970; Wyman and Thrall, 1972; Davis and Brattstrom, 1975; Crovo et al., 2016), their exact purpose is still unknown. Maslin (1950) suggests they are unintentional, but others propose they could serve a purpose in social interactions (Gehlbach and Walker, 1970; Davis and Brattstrom, 1975; Crovo et al., 2016) or orientation (Gehlbach and Walker, 1970). A recent study by Hubáček et al. (2019) registered a high number of underwater low and mid-frequency clicks produced by *Ichthyosaura alpestris* and *Lissotriton vulgaris*, suggesting that newts are much more vocally active than demonstrated by currently available data.

This study tested the hypothesis that the Balkan crested newt *Triturus ivanbureschi* Arntzen & Wielstra, 2013 was vocally active underwater. The aim was to describe the registered sounds and to present possible explanations for the potential role of the produced clicks in the species orientation or interaction.

MATERIALS AND METHODS

Crested newts (*Triturus cristatus* superspecies) are a group of closely related species with medium size (mean adult snout-vent length [SVL] varies between 62.5-84.4 mm for different species, review in Lukanov and Tzankov, 2016) and a biphasic lifestyle (aquatic and terrestrial phase) that are distributed parapatrically in the Old World. The Balkan crested newt is distributed in both Europe and Asia – its range covers the Eastern Balkan Peninsula, including most of Bulgaria, the Eastern parts of Greece, North Macedonia and Serbia, as well as Northwestern Turkey (Speybroeck et al., 2016). In Bulgaria, the species occurs across most of the country from sea level up to 1700 m elevation, inhabiting various types of lentic water bodies (Stojanov et al., 2011). While other newt species (e.g., the Alpine newt *I. alpestris*) have been observed to produce squeaks when handled out of the water or soft gulping sounds when taking air at the water surface (Maslin, 1950), there is no literature data on the sound production of any Crested newt species.

In April 2019 a total of 27 adult newts (21 males, 6 females) were caught using funnel traps in a small natural pond overgrown with bulrush (*Typha* sp.), yellow iris (*Iris pseudacorus*) and common bladderwort (*Utricularia vulgaris*) near the city of Sofia, Bulgaria (42°35'42"N, 23°22'5"E). All animals were housed indoors in a large (100×100×60 cm) glass tank filled with water and were fed common earthworms (*Lumbricus terrestris*) ad libitum. Ambient temperature was constant at 18 °C.

Experiments were conducted in a small glass tank (40×20×60 cm) with water level at 15 cm, with the hydrophone completely submerged 5 cm below the water surface and fixed in such a position as to not touch the bottom or the sides of the tank. Three types of trials were conducted to test for sound production under different conditions – single newt in an empty tank (trial 1), single newt in a tank full of water vegetation (trial 2), and a pair of newts in an empty tank (trial 3). The pair in the third trial would consist of female/female, female/male or male/male. The trials were designed in order to assess whether any registered sounds played a role in orientation or social interaction. After sex determination, newts were placed in the tank individually for the first two trials or as a pair for the third trial, and after 5min of adaptation, newt sounds were recorded for the next 10 min. Snout-vent length (SVL) of all newts was measured with a ruler (to 0.1 cm) and body mass was weighted (to 0.01 g) using digital balance scales (Durascale D2 capacity pocket scale). Because of the crepuscular/nocturnal lifestyle of the study species, all trials were performed in complete darkness. No animals were used in the same trial twice (except for the six females, which were used once in female/female pairs and a second time in female/male pairs) or recorded more than once per day. Vegetation used in the second trial was collected from the study pond and consisted of bulrush and common bladderwort leaves (both freshly cut and old); it covered $^{2}/_{3}$ of the tank, with the hydrophone fixed in the other $^{1}/_{3}$. During the tests there was no human presence in the room and newts had approximately three days to rest between trials. All experiments complied to the international requirements for ethical treatment of animals (Lehner, 1996) and after they were completed, all newts were released at the site of capture.

Vocal activity was recorded using an Olympus LS-5 linear PCM recorder and an omnidirectional Aquarian H2a hydrophone (sensitivity: -180 dB re: 1V/μPa, useful range: 10 Hz to 100 kHz). Recordings were made in a WAV-PCM mode with sampling frequency of 44.1 kHz, 20-21.00 Hz and 24-bit resolution. The recordings were processed with the open source software Soundruler V. 0.9.6.0. (Gridi-Papp et al., 2007). The following parameters were measured: (1) number (the number of clicks produced during each recording), (2) duration (difference between the beginning and the end of a click, measured in ms from the spectrogram), (3) peak frequency (click frequency with the highest energy, measured in Hz), (4) tune 50 (the ratio of peak frequency/bandwidth at 50% peak amplitude), and (5) tune 10 (the ratio of peak frequency/bandwidth at 10% peak amplitude). Spectrograms used for the measurements of click duration had a Hanning window type and an FFT length of 512 points.

All data was tested for normality using a Shapiro-Wilk test and the null hypothesis was rejected ($P < 0.001$). A Mann-Whitney U test was used to compare clicks produced by males and females in the solitary treatment, and a Kruskal–Wallis H test was used to check for differences in the measured parameters between recordings under different conditions. A Spearman rank order correlation was used to test whether SVL and body mass were related to the studied sound parameters. A Kruskal-Wallis H test revealed no statistically significant differences between pairs in trial 3 (see Appendix) and they were treated as one sample for the statistical analyses in the comparison between the trials. The chosen level for statistical significance was P < 0.05. All analyses were carried out using the computer program Statistica v.7.0 (StatSoft, Inc., 2004).

RESULTS

On average, newts started to produce clicks after 173 s (± 122 SD, range: 8-396) and although that latency varied widely between individuals, there were no statistically significant differences across trials (Kruskal-Wallis H test; H(2) = 0.148, P = 0.929) or sexes (Mann-Whitney U test; U = 68.50, P = 0.856). All registered clicks had a simple non-harmonic structure and their peak frequency was in the lower ranges of the spectrum (Fig. 1). Descriptive statistics for all measured parameters are presented in Table 1.

The Mann-Whitney U test revealed there were no statistically significant differences between sexes in all studied parameters of the registered clicks: number (U = 322.500, P = 0.582), duration (U = 621.500, P = 0.473), peak frequency (U = 449.500, P = 0.490), tune 50 (U = 401.500, P = 0.193) and tune 10 (U = 424.500, P = 0.313).

Results from the Kruskal–Wallis H test used for the comparison of the three trials were similar – there were no statistically significant differences among the trials in terms of number (H(2) = 0.443, P = 0.801), duration (H(2) = 5.319, P = 0.070) and peak frequency (H(2) = 3.910, P = 0.142) of the clicks, as well as in parameter tune 10 (H(2) = 2.829, P = 0.243). However, there was a significant difference between the parameter tune 50 from trial 2 and the other two trials - H(2) = 18.067, P = 0.001; values for this parameter were significantly lower in trial 2 compared to trial 1 and trial 3 (Table 1).

Normally clicks were not produced in series, but on three occasions (two in trial 1 and one in trial 2) there were more than one in quick succession. In two of the cases (one in trial 1 and trial 2) the duration of the intervals between the clicks was comparable to that of the clicks themselves at 69 ms and 34 ms, respectively; in the third case, there were three clicks in the space of around one second, with the intervals between them standing at 551 ms and 325 ms.

There was a statistically significant difference in both SVL and body mass between males and females, but there was no significant effect of SVL and body mass on any of the studied parameters (Table 2).

It has to be noted that during handling of the animals, 10 (two females, eight males) out of the 27 newts produced both clicks and squeaks – however, attempted recordings of these sounds were too faint to analyse and therefore were discarded.

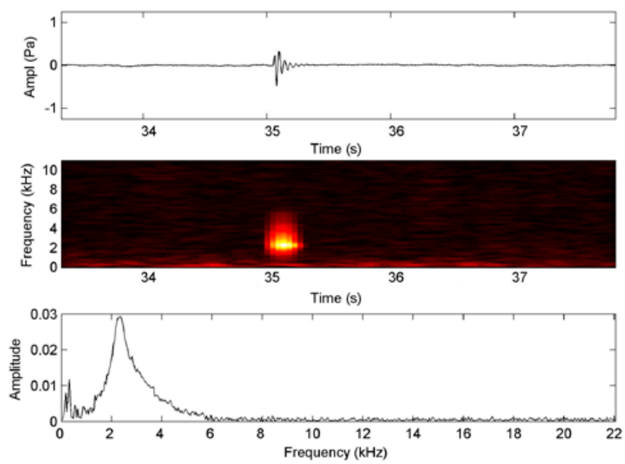

Fig. 1. From top to bottom: indicative oscillogram, spectrogram and amplitude spectrum of a click.

Table 1. Measured parameters of the recorded clicks in *T. ivanbureschi* (number of vocalizing newts/pairs is in parenthesis). Statistically significant differences between trials are in bold; when no such differences are present, a combined value for all groups is given in the last column. Data is presented as Mean (Min-Max ± SD).

	Empty tank (trial 1), n = 27			Vegetated tank (trial 2), n = 27			Pair (trial 3) n = 13 (9)	All groups
	Male (13)	Female (4)	Combined (17)	Male (9)	Female (4)	Combined (13)		
Total clicks	26	4	30	28	11	39	17	86
Number	1.14 (0-4 ± 1.195)	1 (0-2 ± 0.632)	1.111 (0-4 ± 1.086)	1.24 (0-7 ± 2.022)	2.16 (0-8 ± 3.060)	1.444 (0-8 ± 2.258)	1.211 (0-3 ± 1.182)	1.260 (0-8 ± 1.625)
Duration	36 (26-51 ± 5.910)	33 (29-36 ± 2.655)	36 (26-51 ± 5.500)	35 (27-51 ± 6.910)	33 (27-39 ± 3.781)	34 (27-51 ± 6.050)	34 (30-40 ± 2.783)	34 (27-51 ± 5.314)
Frequency	1920 (1392-2656 ± 288)	2096 (1162-2684 ± 510)	1961 (1162-2684 ± 348)	1769 (1397-2346 ± 375)	1785 (1392-2340 ± 379)	1774 (1392-2346 ± 370)	2026 (01162-2770 ± 535)	1887 (1162-2770 ± 405)
Tune 50	9.919 (2.300-26.622 ± 6.709)	6.894 (2.232-11.214 ± 3.751)	**9.221 (2.232-26.622 ± 6.222)**	3.985 (1.599-6.779 ± 1.348)	3.628 (1.625-6.002 ± 1.5508)	**3.877 (1.599-6.779 ± 1.398)**	**8.980 (2.139-16.154 ± 5.146)**	N/A
Tune 10	1.906 (0.494-5.189 ± 1.214)	2.322 (0.527-5.378 ± 2.311)	2.002 (0.494-5.378 ± 1.490)	1.224 (0.646-3.257 ± 0.538)	1.122 (0.644-1.499 ± 0.326)	1.193 (0.644-3.257 ± 0.481)	1.861 (0.485-8.389 ± 2.082)	1.606 (0.485-8.389 ± 1.329)

Table 2. Measurements of SVL and body mass - comparison between sexes and correlations across studied parameters, with statistically significant differences in bold. Data is presented as Mean (Min-Max ± SD).

	SVL (cm)	Body mass (g)
Males	7.75 (7.20-8.70 ± 0.43)	12.01 (7.75-20.35 ± 2.79)
Females	8.3 (7.90-9.10 ± 0.46)	18.57 (12.71-21.42 ± 3.04)
Mann-Whitney U test	**U = 20.00, P = 0.012**	**U = 10.00, P = 0.002**
Spearman rank order correlation test		
Number	r = 0.074, P = 0.592	r = 0.199, P = 0.149
Duration	r = -0.218, P = 0.074	r = -0.074, P = 0.548
Frequency	r = 0.003, P = 0.980	r = -0.002, P = 0.990
Tune 50	r = -0.045, P = 0.733	r = 0.052, P = 0.698
Tune 10	r = -0.110, P = 0.408	r = -0.097, P = 0.466

DISCUSSION

The study demonstrated that the Balkan crested newt emits sounds underwater. Parameters of produced clicks highly overlapped between sexes and across the three test trials, with SVL and body mass also having no visible effect. While underwater sound production has been previously documented in four urodelan families – Ambystomatidae (Wyman and Thrall, 1972; Licht, 1973), Amphiumidae (Crovo et al., 2016), Salamandridae (Davis and Brattstrom, 1975; Hubáček et al., 2019) and Sirenidae (Gehlbach and Walker, 1970) – this is the first report for representatives of the Crested newts species group, and the second on European salamandrid species, following the recent study of Hubáček et al. (2019). Wien et al. (2011) assert that sound-producing lineages of salamanders have split from their common ancestors around 60 mya, which indicates that sound production is either a shared trait among newts, or has evolved independently in several lineages. Steinfartz et al. (2007) estimate that the last common ancestor of the *Triturus* species group lived around 64 mya, which is a very similar timeline. The latter study established that the *Triturus* assemblage included four monophyletic groups: large-bodied newts (incl. *T. ivanbureschi*, referred to as "*T. karelinii*"), small-bodied newts (incl. *L. vulgaris*, study species in Hubáček et al., 2019), *I. alpestris* (study species in Hubáček et al., 2019) and *Ommatotriton vitatus*. This early separation between large-bodied Crested newts and the smaller species of the assemblage offers at least partial explanation for the differences between the produced sounds. Hubáček et al. (2019) established two types of clicks (low frequency and mid-high frequency), but found no difference between clicks produced by *L. vulgaris* and *I. alpestris*. Their mean duration varied between 7.67 ms and 10.74 ms for the low clicks and between 10.60 ms and 12.14 ms for the mid-high clicks, which is around three to five times shorter than the clicks recorded in the present study. The low-frequency clicks had a mean peak frequency between 7.46 kHz to 7.97 kHz, which is still significantly higher than the average of 1.89 kHz established for *T. ivanbureshi*. In any case, direct comparisons should be considered with caution, as some of the differences could be due to the different equipment and settings used for the recordings.

Nevertheless, it has to be noted that clicks in *T. ivanbureschi* were more similar to the ones in the American salamandrid species *Taricha torosa*, which had duration between less than 0.1 s up to 0.4 s and frequency of 1.4-8 kHz (Davis and Brattstrom, 1975). Results of the present study also seem to be consistent with the authors' observation that clicks in *T. torosa* were produced during exploratory behaviour, when newts were placed in an unfamiliar setting; however, additional studies are needed in order to clarify this. Both species are of similar size and overall body shape (in contrast to *L. vulgaris* and *I. alpestris*, which are smaller and less robust), but at present more studies are needed in order to establish whether phenotypic similarities could result in production of similar sounds. Unlike *T. ivanbureschi*, clicks produced by *T. torosa* did have a harmonic structure, with harmonics at 3.5, 5 and 6.5 kHz (Davis and Brattstrom, 1975). While there was only one statistically significant difference between trial 2 and the other two trials (parameter tune 50), this and the relatively higher number of recorded clicks in trial 2 could implicate the role of sound in *T. ivanbureschi* orientation. For some anurans, call parameters such as call rate and pulse number have been established to have a highly directional effect (Gerhardt, 1991) and this could also be true for newts, with the dynamic ratio of frequency/amplitude being beneficial in orientation. While data for definitive conclusions is still lacking, there are studies demonstrating that acoustic information in the form of anuran calls improves orientation in other newt species (e.g., Diego-Rasilla and Luengo 2004, 2007). The only other study on sound production in caudate amphibians that also provides data for body mass is that of Hubáček et al. (2019), which also did not find any relation between weight and produced clicks, so the possible role of SVL and body mass on sound production in newts remains to be clarified. The fact that some of the newts used in this study produced clicks while being handled out of the water could indicate that clicks are some form of a distress signal, or displacement activity (involuntary response) – the latter has been demonstrated for visual signals in some anuran species (Furtado et al., 2016); however, since recordings of terrestrial clicks could not be analysed, it is yet unclear if they are identical to the ones registered underwater.

Wyman and Thrall (1972) report two types of clicks for *Ambystoma maculatum,* with mean frequency and duration around 1.5 kHz/0.09 s and 0.5-6.5 kHz/0.04 s, respectively. The authors state that while the behavioural significance of both sounds was unknown, the lower frequency click was only recorded during the breeding season, suggesting a possible role in reproduction. Considering that April is peak breeding season for *T. ivanbureschi*, such a possibility cannot be excluded, but it is still not very likely, as none of the pairs in trial 3 produced significantly more clicks. However, juveniles or adult newts outside of the breeding season were not tested in the present study, so additional research is needed in order to establish the potential role of age and phase in sound production for this species. For the congeneric species of *A. gracile*, Licht (1973) describes sounds that are very similar to those of *A. maculatum* (peak frequency 0.4-2.5 kHz, duration 0.04-0.06 s) and suggests that they are associated with both defensive and aggressive behaviour. Again, this seems unlikely for *T. ivanbureschi*, as in trial 3 there were no signs of antagonistic behaviour.

While Crovo et al. (2016) report both low- and high-frequency clicks in *Amphiuma means* as having a role in social interaction, they admit that "the high-frequency clicks produced, however, were not associated with high-frequency hearing" – i.e., their behavioural significance remains unclear. Low-frequency clicks were associated with communication in *Siren intermedia*, but the authors also suggest that the acoustic signals may play a role in orientation when visual and olfactory cues are absent (Gehlbach and Walker, 1970). Both of the abovementioned species are exclusively aquatic, while *T. ivanbureschi* has a pronounced biphasic lifestyle, meaning that underwater sound communication could be expected to be less complex in this species.

While results for the sounds produced by *T. ivanbureschi* are not conclusive, they imply that sound plays a certain role in this species behaviour. It has to be said that sound production in caudate amphibians is likely to prove a more productive area for scientific research than previously thought - even the few existing studies so far exhibit a large variation of results and different interpretations. Crested newts in particular could be suitable study species because of their well-studied phylogeny, allowing for better inter-species comparisons.

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APPENDIX

Results of the Kruskal-Wallis H test for comparison of the studied parameters between newt pairs (female/female, female/male, male/male), n = 13.

- Number of clicks: H(2) = 0.952, P = 0.621
- Duration of clicks: H(2) = 4.532, P = 0.104
- Peak frequency: H(2) = 0.344, P = 0.842
- Tune 50: H(2) = 0.557, P = 0.757
- Tune 10: H(2) = 0.524, P = 0.769