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Does size matter? – Reproductive success in *Duttaphrynus melanostictus* as a function of body size

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ABSTRACT. – We examined the mating pattern in the bufonid species *Duttaphrynus melanostictus* at a site in northeastern Thailand. During a breeding aggregation we determined snout–vent length (SVL) and body weight of amplectant males and females as well as of non-amplectant males. Additionally, we calculated the ratio of weight/SVL. In these parameters, there were no statistically significant differences between amplecting and non-amplecting males. We found a significant, moderate positive correlation for amplectant males and females SVL, indicating that larger females tend to mate with larger males. Thus, this positive correlation between the body sizes of mating partners is interpreted as evidence for size-assortative mating. We also provide a description of the advertisement call of this species from our study site.

KEYWORDS: amphibians, breeding ecology, mating pattern, reproduction, Southeast Asia

INTRODUCTION

The Asian common toad (*Duttaphrynus melanostictus*) is distributed widely across South and Southeast Asia. It is commonly found in disturbed habitats and actually thrives best in human-modified environments. Recent studies documented several genetic lineages suggesting that *D. melanostictus* actually represents a species complex (Wogan et al. 2016).

In species with very short breeding periods, such as is the case for *D. melanostictus*, many individuals arrive at the breeding site simultaneously. In these explosive breeders, male mating success is determined by direct male-male competition for individual females. Three basic mating patterns can be distinguished in most bufonid species, (1) a random mating pattern; (2) a large male mating advantage; and (3) size assortative mating (Davies and Halliday 1979; Olson, Blaustein and O'Hara 1986; Yu and Lu 2010). In species with a large male mating advantage (i.e., *Bufo bufo, B. gargarizans*), larger males had greater reproductive success because their greater strength made them more able to displace other males from the backs of females (Davies and Halliday 1979; Lu and Liao 2011). In other words, large male mating advantage is caused by competition among males rather than by female choice (Lu and Liao 2011).

A male of an amplecting pair with the partners differing strongly in size is more easily displaced by another male than in pairs that more closely match in size (Höglund 1989). Evidence for the occurrence of size-assortative mating was found for some bufonids (Höglund 1989; Vogrin and Miklič 2005). Other studies have indicated random mating patterns in bufonid species (Wells 1979; Yu and Lu 2012). Finally, for some species in the family Bufonidae, intraspecific variation of the mating patterns have been documented (Olson, Blaustein and O'Hara 1986), and even cases where males found in amplexus were larger on average than non-amplectant males (i.e., large male mating advantage) and the amplecting partners had an optimal size ratio (i.e., size assortative mating) (Yu and Lu 2010). Factors responsible for intraspecific variation in mating patterns include breeding site conditions (e.g., size of water bodies, elevation above sea level), operational sex ratios, and individual densities of toads (Olson, Blaustein and O'Hara 1986; Liao and Lu 2012).

Gramapurohit & Radder (2012) studied the mating pattern in *Duttaphrynus melanostictus* at a site in India and provided evidence for size-assortative mating and also found that amplecting males were larger in size than their non-amplectant competitors. Size-assortative mating was confirmed for this species by Fan et al. (2013) who studied a population in eastern China. In male mate choice experiments with *D. melanostictus*, males did not show preferences for larger females and the authors concluded that male mating success in this species is likely determined by the number but not the quality of mates (Zhang et al. 2020).

In this study we examined the mating pattern in the bufonid species *Duttaphrynus melanostictus* at a site in northeastern Thailand. We also provide a description of the advertisement call of this species from our study site.

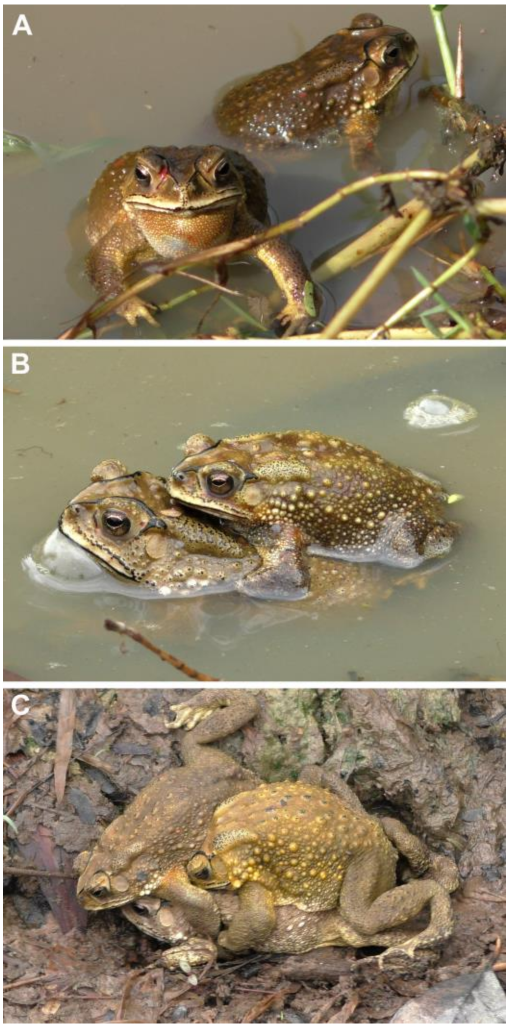

FIGURE 1. *Duttaphrynus melanostictus* in life. A. Vocalizing males; B. Male and female in amplexus; C. Two males clinging to a female. Photos by G.K.

MATERIALS AND METHODS

Field work in Thailand for this study was done by GK and PT during March through July 2020. In the morning hours of 24 and 25 May 2020 after substantial rain during the preceding nights, GK encountered numerous calling males of *Duttaphrynus melanostictus* at the edge of a large pond as well as amplecting pairs of these toads in the shallow water (Fig. 1). The study site is on the edge of the village of ban Sa At Na Di (16.16256, 104.11349; WGS 1984 datum), 150 m a.s.l., Roi Et province, Thailand. By 11:00 am on both days most toads had left the water body and disappeared. Numerous gelatinous egg strings were present in the shallow water of the pond. GK measured SVL of 37 males and 26 females during these two days. 26 of these males have been in amplexus with a female whereas 11 were caught while they were still single. Of each toad snout–vent length (SVL) and weight was determined. The toads were kept in big buckets until all individuals had been measured and weighted to prevent taking data from the same individual twice. In addition the ratio weight/SVL was calculated as a proxy for the nutritional condition. Abbreviations used: FA = Female Amplexus; MA = Male Amplexus; MNA = Male No Amplexus; SD = standard deviation; SVL = snout–vent length.

We preserved two adult males (field numbers GK-7493, 7495) and two adult females (GK-7492, 7494) of *Duttaphrynus melanostictus* as voucher specimens. These were deposited in the collections of Senckenberg Forschungsmuseum Frankfurt (SMF) and Chulalongkorn University, Museum of Natural History (CUMZ), Bangkok. We euthanized the toads with a pericardial injection of T61. We cut tissue samples from the tongue and preserved these in 98% non-denatured ethanol for DNA extraction. The tissue samples were deposited in the collection of SMF and CUMZ. Specimens were then preserved by injecting a solution of 5 mL absolute (i.e., 36%) formalin in 1 L of 96% ethanol into the body cavity, and stored in 70% ethanol. Coordinates and elevation were recorded using Garmin GPS receivers with built-in altimeters.

We recorded anuran vocalizations using a digital audio recorder (Olympus LS-12) with a Sennheiser ME 66 shotgun microphone capsule and a Sennheiser K6 powering module. The microphone was positioned between 0.5 and 1.5 m from the calling frog. Aside from the GPS coordinates and elevation above sea level of the locality we also noted ambient air temperature and determined SVL of the recorded individual. Files were recorded as uncompressed 24-bit WAV files at a sampling frequency of 96 kHz. Audio files were deposited in the Fonoteca Zoológica, Museo Nacional de Ciencias Naturales, Madrid, Spain.

The spectral and temporal parameters were analyzed and the power spectra were calculated in RAVEN PRO 1.4. (Bioacoustics Research Program 2011). Spectrograms were obtained using the Blackman window function at 3db Filter Bandwidth of 158 Hz; grid spacing of 21.5 Hz; overlap 90%. Frequency information was generated through Fast Fourier Transformation (FFT, width 2,048 samples). Temporal measurements of calls such as repetition rates, duration of notes, and number of pulses, were measured on the waveforms. Terminology in call descriptions follows (Köhler et al. 2017).

Descriptive statistics (range, mean value and SD) were calculated using Microsoft Excel. The obtained data were analyzed using RStudio 1.2.1335 (R Studio Team 2018). A Shapiro-Wilk test was performed using the package "RcmdrMisc" version 2.7-1 (Fox 2020) and "nortest" version 1.0.-4 (Gross and Ligges 2015) to test the data for normality. The homogeneity of variances was tested using the Levenne test. Therefore the package "car" version 3.0.7 was used (Fox and Weisberg 2019).

First, we analyzed differences between sexes in snout-vent-length (SVL), body weight and ratio weight/SVL. For data that were normally distributed and variance homogeneous, a Student's t-test was performed using the package "stats" version 3.6.2 (R Core Team 2019). In case the data were normally distributed and variance inhomogeneous, a Student's t-test for invariant data was performed. In case the results were significant, another Student's t-test was calculated to see if the females had higher scores than the males.

Because the body weight data were not normally distributed, a Wilcoxon test was performed using the package "stats" version 3.6.2. (R Core Team 2019). If the results were significant, another Wilcoxon test was calculated to see if the females had higher scores than the males.

We then examined the differences between amplecting and non-amplecting males in terms of SVL, weight and ratio weight/SVL. Since the SVL data were normally distributed and variance homogeneous, a Student's t-test was again performed. Body weight and ratio weight/SVL were not normally distributed. Therefore, a Wilcoxon test was performed.

The parameters SVL, body weight and ratio weight/SVL of amplectant *Duttaphrynus melanostictus* males and females were checked for size assortative mating. Since SVL was normally distributed, a Pearson's product moment correlation coefficient was calculated using the package "stats" version 3.6.2 (R Core Team 2019). The same package was used to calculate a Spearman's rank correlation for body weight and ratio weight/SVL since these two parameters were not normally distributed.

The relationships of amplectant males and females SVL, weight, and ratio weight/SVL were plotted using scatterplot (package "car" version 3.0-7) and a regression line was drawn using the regLine argument (package "car" version 3.0-7) (Fox and Weisberg 2019).

RESULTS

The variation in the studied parameters is shown in Table 1. The females (all from amplecting pairs) had a SVL of 77–102 mm (89.73±6.30) and a weight of 35.8–99.7 g (68.77±16.99) whereas the males (amplecting and non-amplecting combined) had a SVL of 63–95 mm (79.89±6.96) and a weight of 21–74.4 g (43.59±12.48). The amplecting males had a SVL of 68–95 mm (80.65±6.80) and a weight of 25.8–74.4 g (45.55±12.85) whereas the non-amplecting male had a SVL of 63–89 mm (78.09±7.33) and a weight of 21.0–56.9 g (38.97±10.72). Also, in the ratio weight/SVL, a similar pattern was found: females 0.459–1.035 (0.761±0.160), amplecting males 0.379–0.798

TABLE 1. Range and mean ± SE for the groups (females; males total; amplecting males; non-amplecting males) in relation to SVL [mm], weight [g] and ratio weight/SVL. N representing number of *Duttaphrynus melanostictus* individuals used for this study.

Group (N)	SVL [mm]	Weight [g]	Ratio weight/SVL
Females (26)	77–102 (89.73±6.30)	35.8–99.7 (68.77±16.99)	0.459–1.035 (0.761±0.160)
Males total (37)	63–95 (79.89±6.96)	21–74.4 (43.59±12.48)	0.333–0.798 (0.537±0.109)
Amplecting males (26)	68–95 (80.65±6.80)	25.8–74.4 (45.55±12.85)	0.379–0.798 (0.557±0.110)
Non-amplecting males (11)	63–89 (78.09±7.33)	21–56.9 (38.97±10.72)	0.333–0.639 (0.491±0.097)

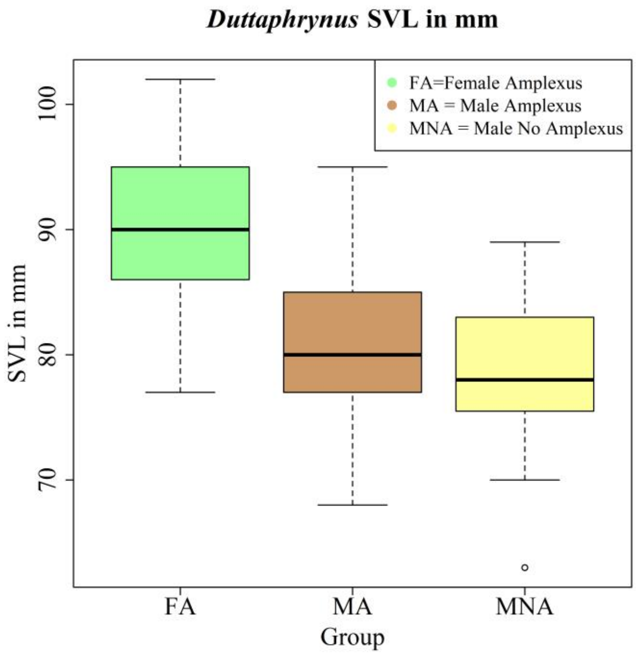

FIGURE 2. Variation of SVL in mm of the *Duttaphrynus* specimens

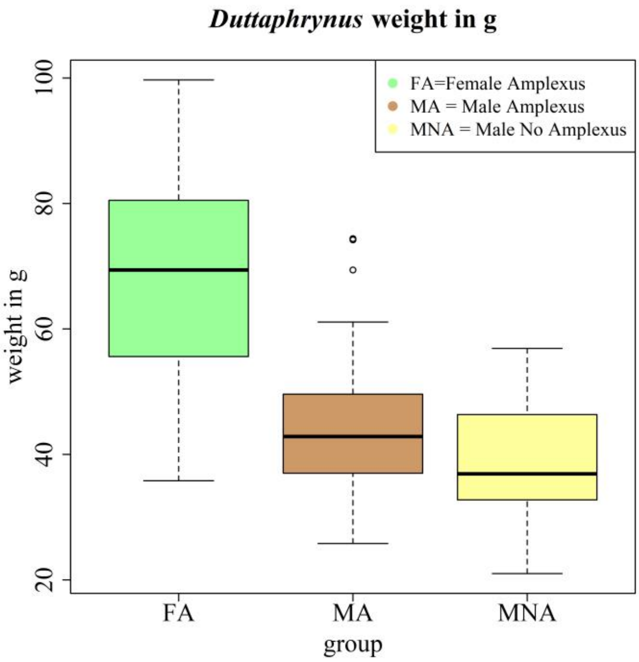

FIGURE 3. Variation of weight in g of the *Duttaphrynus* specimens

(0.557±0.110), and non-amplecting males 0.333–0.639 (0.491±0.097).

Statistical analysis of the data between the sexes revealed the following: SVL and the ratio weight/SVL data showed normal distribution whereas those for weight did not. For variation in SVL and the ratio weight/SVL the Student's t-test revealed significant differences between the males and females (SVL: $p<0.01$; weight/SVL: $p<0.01$). Females are significantly larger (SVL: $p<0.01$; Fig. 2) and have a significantly larger weight/SVL ratio ($p<0.01$; Fig. 4) than males.

For variation in body weight the Wilcoxon test showed significant differences between males and females ($p<0.01$). Females are significantly heavier than males ($p<0.01$; Fig. 3).

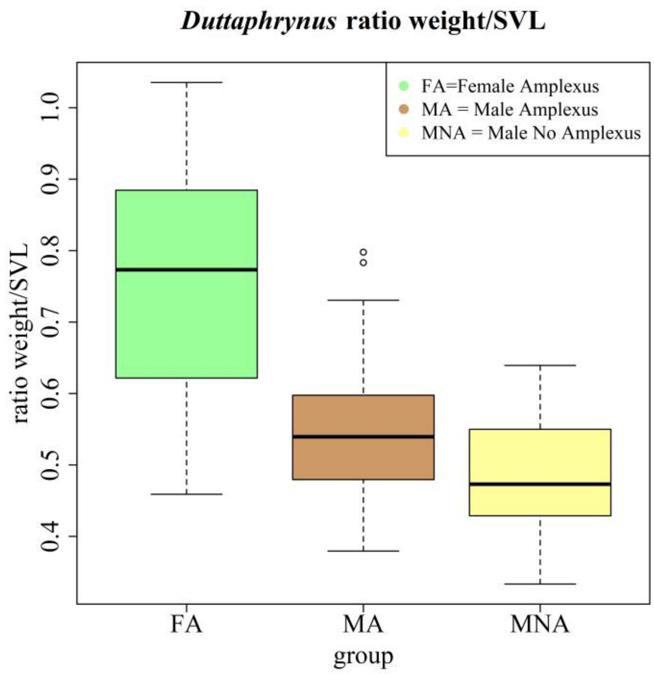

FIGURE 4. Variation of the ratio weight/SVL of the *Duttaphrynus* specimens

TABLE 2. Results of Pearson's product moment correlation coefficient and Spearman's rank correlation of SVL, body weight, and the ratio weight/SVL between *D. melanostictus* males and females. Descriptive statistics represented by range, mean value and SD. Pearson's product moment correlation coefficient variables: N = Number of *D. melanostictus* individuals used for this analysis, r = Pearson's correlation coefficient, rho = Spearman's coefficient, *p* = significance, significant p-values in bold.

Parameter	Amplected females	Amplecting males	N	r/rho	*p*
SVL [mm]	77–102 (89.73±6.30)	68–95 (80.65±6.80)	26	0.4093442	**0.03784**
Weight [g]	35.8–99.7 (68.77±16.99)	25.8–74.4 (45.55±12.85)	26	0.3843392	0.05255
Ratio weight/SVL	0.459–1.035 (0.761±0.160)	0.379–0.798 (0.557±0.110)	26	0.2574359	0.2034

In contrast, statistical analyses between amplecting (MA) and non-amplecting (MNA) males revealed the following: SVL data showed normal distribution whereas those for body weight and ratio weight/SVL did not. For variation in SVL the Student's t-test did not reveal significant differences (SVL: p = 0.334). In addition, the Wilcoxon test did not show significant differences in body weight (p = 0.1839) and the ratio weight/SVL (p = 0.1107). There are no statistically significant differences between amplecting and non-amplecting males in these parameters (Figs. 2–4).

The results of the Pearson's product moment correlation coefficient are shown in Table 2. We found a significant, moderate positive correlation for amplectant males and females SVL (r = 0.40934, p = 0.03784), indicating that larger females tend to mate with larger males (Fig. 5). The Spearman's rank correlation for body weight (r = 0.38434, p = 0.05255; Fig. 6) and ratio weight/SVL (r = 0.25744, p = 0.2034; Fig. 7) show no significant correlation for amplectant males and females.

The advertisement call of *Duttaphrynus melanostictus* at our study site (recorded on 24 May 2020 at 9:00 h; ambient temperature 29°C) is a trill with the following characteristics (see also Fig. 8): Call length 3.3–11.4 s (6.6±2.52 s); dominant frequency 1249–1314 Hz (1273±15.9 Hz); pulse length 60–75 ms (67±0.45 ms); 8.11 pulses per second.

DISCUSSION

Our results show that none of the studied parameters were significant different between amplecting and non-amplecting males. Thus, body size is not a factor that has a measurable effect on mating

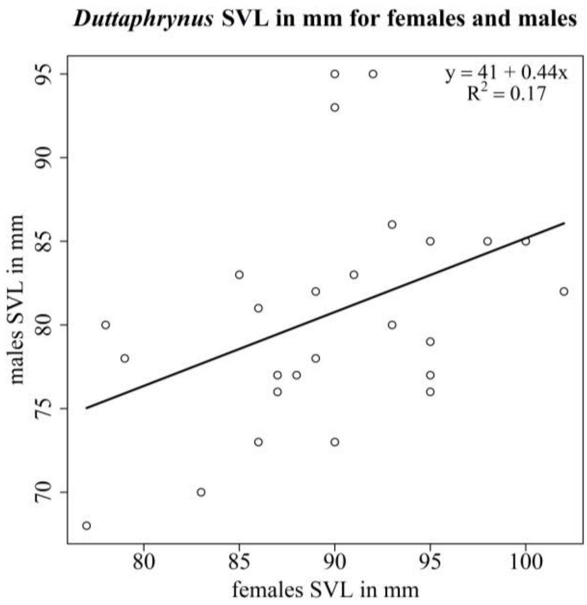

FIGURE 5. Relationship SVL in mm of amplecting females and males. The Pearson's product moment correlation coefficient revealed a significant, moderate positive correlation for amplectant males and females SVL (r = 0.40934, p = 0.03784).

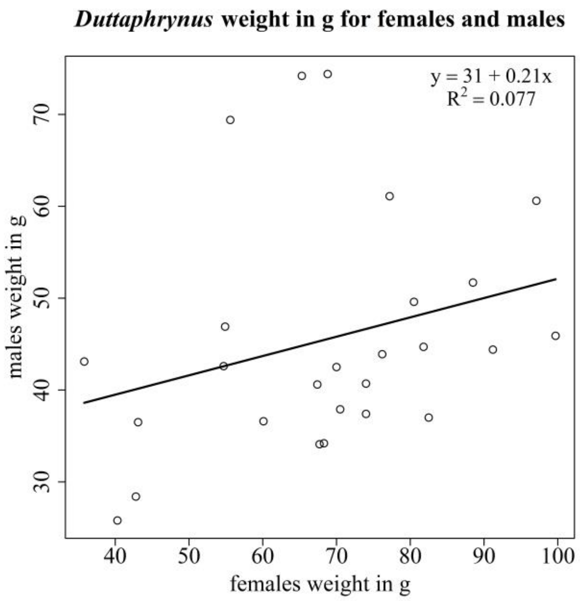

FIGURE 6. Relationship body weight in g of amplecting females and males. The Spearman's rank correlation for body weight revealed no significant correlation for amplectant males and females (r = 0.38434, p = 0.05255).

success in the *Duttaphrynus melanostictus* studied here. The adult males have strong forelimbs and well-developed nuptial pads that enables them to grab and hold onto the females for amplexus. Thus, we found no evidence for a large male mating advantage. Obviously, mating success is mostly determined by luck, i.e., whichever male happens to be near enough to a non-amplecting female to be able to grab it. Once in amplexus, the male vigorously defends its female against other males that try to mount it. Therefore, which male the female toad ends up in amplexus with is not a matter of female choice. These findings disagree with Gramapurohit & Radder (2012) who found that amplecting males in *D. melanostictus* were larger in size than the non-amplectant individuals studied. Our data indicate size assortative mating in *D. melanostictus* at our study site which is congruent with the results of Gramapurohit & Radder (2012) and Fan et al. (2013) who studied the same species in India and China, respectively. In our data set, larger males paired

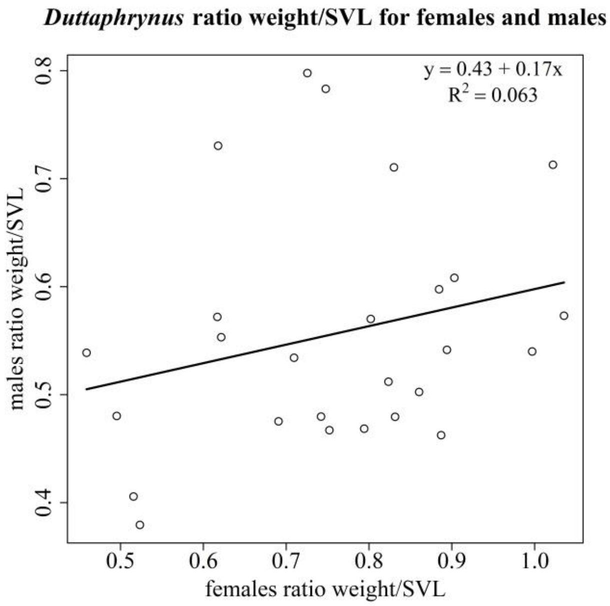

FIGURE 7. Relationship ratio weight/SVL of amplecting females and males. The Spearman's rank correlation for ratio weight/SVL revealed no significant correlation for amplectant males and females (r = 0.25744, p = 0.2034).

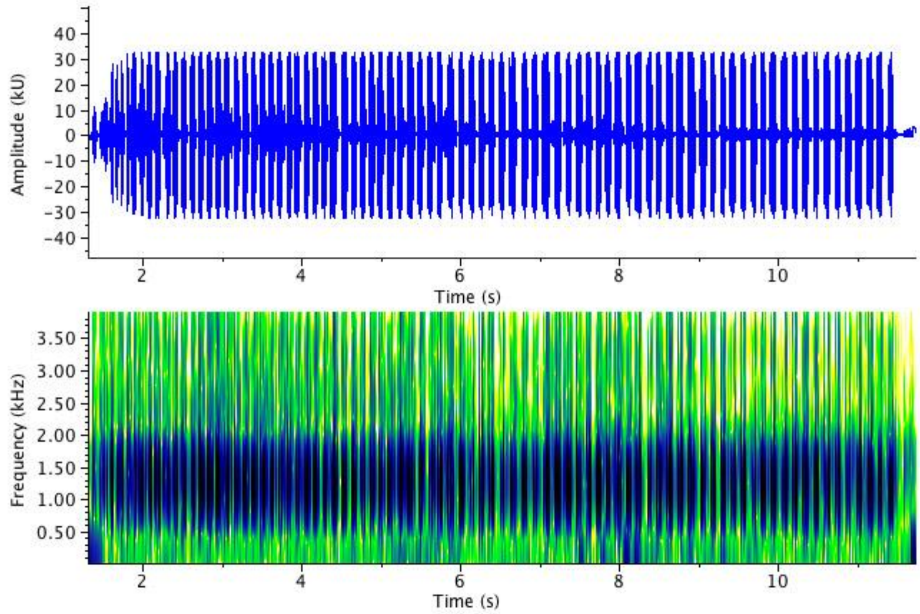

FIGURE 8. Advertisement call of male *Duttaphrynus melanostictus*. See text for details

with larger females and smaller males with smaller females. Thus, this positive correlation between the body sizes of mating partners is interpreted as evidence for size-assortative mating. The reason for this phenomenon most likely is that for a male in amplexus with a female of about the same size it is easier to hold on to the female when it has to defend its amplexus position against competitive males. A male that is much smaller than the female it has mounted on the other hand does not achieve a strong hold on the female and is easier replaced by another male (Gramapurohit and Radder 2012).

We observed several females with more than one male clinging to it. In two cases, it led to drowning of the female. This behavior in which males do not distinguish between sexes occurs also in other toad species, e.g., *Bufo bufo* (Sztatecsny et al. 2006). Also, males of *Bufo bufo* sometimes even try to mate with heterospecific species or non-living items (Marco & Lizana 2002). To avoid male-male matings, the males of most bufonid species emit a release call and try to detach from each other by vigorous kicking (Khan & Koo 2017). Based on our observations, this is also the case in *D. melanostictus*.

In conclusion, *D. melanostictus* male mating success is mostly determined by the number of available female partners but not by the size of the males.

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