

The effect of size-assortative mating on fertilization success of the common toad (*Bufo bufo*)

Petr Chajma*, Jiří Vojar

Abstract. Although size-assortative mating (SAM) is a frequently studied phenomenon in anurans, its effect on fitness rarely has been evaluated. Using a controlled experiment, we assessed the presence of SAM in the common toad, *Bufo bufo*, and evaluated the effect on fertilization success of male-female size (snout-vent length, SVL) difference and ratio in mated pairs. Even though the toads paired non-randomly with respect to size, the difference and ratio of female and male SVL in pairs had no significant effect on fertilization success. Our findings and the majority of other studies suggest that the commonly accepted idea that SAM serves to maximize fitness may not be completely accurate. The statistical power and effect size in these studies are often poor, however, and the methods used are inconsistent. We conclude that more manipulative experiments with appropriate sample sizes are needed to fully understand this phenomenon.

Keywords: clutch size, explosive breeders, fertilization rate, fitness, non-random mating, sexual selection, size-dependent mating.

Introduction

Non-random or assortative mating is defined as a correlation of certain characteristics of males and females in pairs (Lewontin, Kirk and Crow, 1968). This phenomenon has been documented in a wide spectrum of invertebrates (Brown, 1993; Hegde and Krishna, 1997; Hargeby and Erlandsson, 2006) and vertebrates (Arak, 1983; Mckaye, 1986; Rintamäki et al., 1998; Shine et al., 2001). Characteristics of paired individuals commonly examined by correlation analysis include body mass (e.g., Márquez and Tejedo, 1990), age (Ferrer and Penteriani, 2003), physical condition (Bortolotti and Iko, 1992), and social experience (Freeberg, 1996). The most studied, however, is size-assortative mating (SAM). This is probably due to the fact that size is commonly measured throughout the animal kingdom (Crespi, 1989; Andersson, 1994), including for amphibians (Arak, 1983; Wells, 2007).

Among amphibians, SAM has been found in only anurans and the attention has been focused mainly upon the ranid and bufonid families (e.g., Davies and Halliday, 1977; Elmberg, 1987; Marco et al., 1998; Lu et al., 2009). The reports of its occurrence differ not only among species, but also within populations of the same species and even between breeding seasons. Some studies might report false positive or negative results as a result of incorrectly evaluating the correlation tests used in examining SAM's occurrence. False positive results are often the consequence of relying solely on the test of significance and not taking into account the effect size. Small sample size might, on the other hand, can cause false negative results (Fisher, 1921; Zar, 1984; Vojar et al., 2015).

Moreover, despite the considerable attention that has been given to SAM, only a few studies have moved beyond examining the presence or absence of SAM and undertaken to explain the causes and effects of this phenomenon. It is believed that SAM can increase fitness (Burley, 1983), because pairs consisting of partners well matched for size should also have a higher proportion of fertilized eggs (Davies and Halliday, 1977; Crespi, 1989). This is achieved due to a

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smaller distance between cloacae and a lesser chance of not noticing the signal for the start of egg deposition, which is usually provided by the female in the form of a leg bump (Wells, 2007). Furthermore, size-assortatively formed pairs could have better chances of withstanding the attacks of single males (Davies and Halliday, 1977; Lu et al., 2010).

The only studies which have found a positive effect of SAM on fitness have been those of Ryan (1983) in the tungara frog (*Engystomops pustulosus*) and Bastos and Haddad (1996) in the elegant forest treefrog (*Dendropsophus elegans*). Nevertheless, some studies accept it as a fact (e.g., Davies and Halliday, 1977; Yu and Lu, 2010). Using a controlled experiment, we therefore examined the presence of SAM in the common toad, *Bufo bufo*, and the effect of SAM on one component of fitness, i.e., the proportion of fertilized eggs. Specifically, we evaluated the effect of male and female body size difference and ratio within paired toads on clutch fertilization success.

The common toad is a temperate European toad with a breeding period approximately one month long (Wells, 1977; Davies and Halliday, 1979). The majority of mating occurs, however, in the short period of the main reproduction season, which can last for about a week (Vojar et al., 2015). The toad is therefore regarded as a typical explosive breeder (Arak, 1983). Within such species, males fight for the possession of females, and the mating pattern depends upon male density at the breeding site (Arak, 1983; Wells, 2007), because increasing male-male competition should enhance the intensity of both size-dependent (Davies and Halliday, 1979) and size-assortative (Vojar et al., 2015) mating within a population. The presence of size-dependent mating has been frequently documented within the species (e.g., Davies and Halliday, 1979; Höglund and Säterberg, 1989; Arntzen, 1999; Reading, 2001), as has been the occurrence of SAM (reviewed in Vojar et al., 2015). With an explosive breeder, it is easier to set up natural breeding conditions and control

the experiment. A well-documented explosive breeder such as the common toad is therefore a good model species for this type of experiment.

Materials and methods

Experimental design

The experiment was carried out at artificial ponds on the campus of the Czech University of Life Sciences in Prague. The toads had been captured using pitfall traps and barrier fencing as part of a rescue transfer of amphibians which were attempting to migrate across roads near Hradec Králové, a city in eastern Bohemia, during the spring of 2013. Due to the strong migration, all the amphibians were collected in a single day. The collected toads were first examined for presence of the pathogen *Batrachochytrium dendrobatidis* according to a well-regarded methodology (Hyatt et al., 2007). Sampling was performed by a non-destructive method of skin swabbing using the sterile Dryswab[®] product (MW100, Medical Wire & Equipment Co, UK). Swab samples were processed using DNA isolation by PrepMan Ultra, and *Bd* detection was performed by real-time quantitative PCR (Boyle et al., 2004) with the addition of bovine serum albumin (BSA) to limit PCR inhibition (Garland et al., 2010). All captured toads were free of the pathogen.

To screen captured individuals, 120 males and 79 females were measured using a plastic calliper to obtain snout to vent length (SVL) to the nearest millimetre and weighed using a digital scale to the nearest 0.1 g. These measurements were then used to compare SVL and mass of males and females. In order to simulate a naturally male-biased operational sex ratio of toads at approximately three males to one female (Davies and Halliday, 1979; Reading, 2001; Brede and Beebee, 2006; Vojar et al., 2015), 120 males and 43 females were chosen for the experiment and moved into a single outdoor breeding pool of size 400 × 400 × 100 cm [length × width × height]. There, they were permitted to interact freely. The breeding pool was designed to be as close to a natural pond as possible, i.e. natural vegetation, size of the water body and a mild slope of the banks. Pairs were considered final if there was no successful male displacement for 15 minutes. After approximately one hour, all of the females had paired with a final male. Pairs were moved into separate containers of dimensions 37 × 24 × 26 cm, filled with aged tap water. The pairs were then permitted such time as they needed to reproduce.

After clutch deposition and fertilization, all paired males and females were measured in order to determine the presence of SAM. Thirty randomly chosen clutches were then moved into a specially designed permeable container made out of a polystyrene foam ring and nylon stockings (see Vojar, Doležalová and Solský, 2012) and into hatching pools of size 70 × 150 × 50 cm, filled with the same aged tap water. All the eggs therefore developed under the same environmental conditions in terms of water quality, temperature, shading, etc. In order to distinguish unfertilized and developing eggs, and to determine the proportion of fertilized

eggs, clutches were transferred to white plastic trays with a small amount of water after reaching Gosner stage 16-20 (Gosner, 1960). All eggs were photographed in the trays and then carefully counted from the photographs.

To determine whether mating success of males was affected by their size (Arak, 1983), 28 randomly chosen single males and the same number of mated males were measured. To test the hypothesis that pairs consisting of partners well matched for size should also have a higher proportion of fertilized eggs (Davies and Halliday, 1977; Crespi, 1989), we used both the difference between and ratio of male and female SVL in pairs. Both of these obviously correlated parameters were used because both have been used in similar studies and we wanted to compare our results with the others. Furthermore, we tested if fertilization success depended on male SVL directly, which is highly correlated with testis size (Emerson, 1997).

Data analysis

Mean SVL of mated and unmated males were compared using a Student's *t*-test. Presence of SAM was tested using the non-parametric Spearman's correlation index, because the assumption of normal distribution of both male and female SVL was not met. The possible effect of difference and ratio of male and female body sizes in pairs, as well as of male SVL, on fertilization success could not be tested using a multiple linear regression model due to strong correlation among explanatory variables. To compensate for that, three simple linear regression models were constructed separately. Although this approach is also not entirely correct, because the effects of explanatory variables are overlapping, it did provide valuable insight. Due to heteroscedasticity of errors, dependence of clutch size on female SVL was tested by simple linear regression using heteroscedasticity consistent standard errors, or HC3, which is suggested for small samples (Long and Ervin, 2000). Statistical power and the effect sizes (Cohen's *d*, *r* and f^2) were calculated according to Cohen (1988). All statistical analyses were performed in R software, version 3.2.3 (R Development Core Team, 2015).

Results

Body size of paired males (mean = 62.11, SD = 4.93) was significantly larger than that of single males by approximately 4.32% ($t = 2.21$, $df = 54$, $p = 0.02$; Student's *t*-test) with the mean difference of 2.57 mm (0.24-4.9, 95% CI, two-tailed test) and a medium-large effect size (Cohen's $d = 0.59$), thus indicating size-dependent mating. There was a significant positive correlation between male and female SVL in pairs, indicating the presence of weak SAM ($r = 0.33$, $n = 43$, $p = 0.03$; Spearman's rank

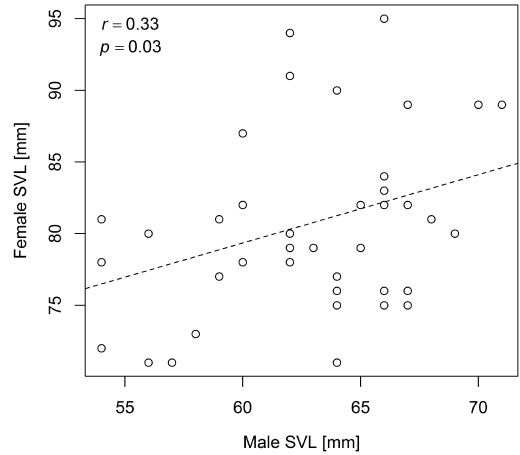


Figure 1. Correlation of body sizes (snout-vent length in mm, SVL) between the common toad males and females in pairs.

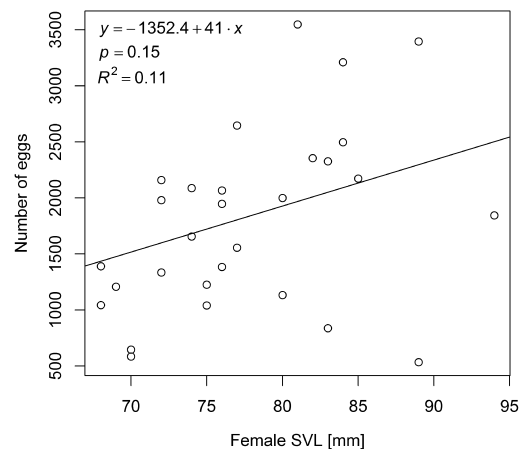


Figure 2. Relationship between clutch size (number of eggs) and female body size (female snout-vent length in mm, SVL) of the common toad.

correlation, fig. 1), with a relatively wide 95% CI (0.04-0.58).

Clutch size (the sum of developed and undeveloped eggs) was marginally independent of female body size ($t = 1.9$, $p = 0.07$). After accounting for an obvious heteroscedasticity issue (fig. 2), the effect appeared even less significant ($t = 1.47$, $p = 0.15$, $R^2 = 0.11$). Although the non-significant relationship could be caused by the lack of statistical power, the effect size (Cohen's $f^2 = 0.12$) was still low. With the given sample size and proper power (0.8), we would

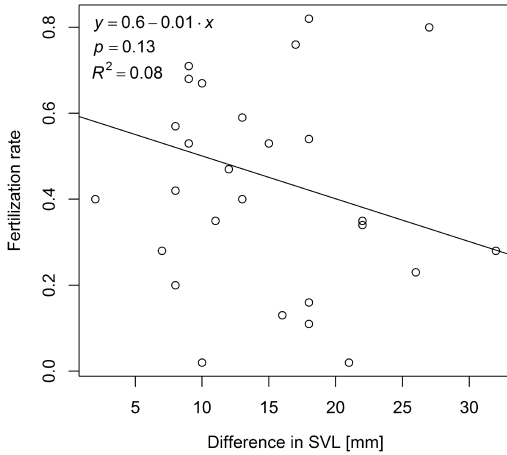


Figure 3. Relationship between fertilization success and difference in female and male body sizes (difference in snout-vent length in mm, SVL) of the common toad.

be able to detect only a medium to large effect ($R^2 \geq 22\%$; Cohen, 1988). Both the total number of eggs (534–3548) and fertilization rate (2–89%) varied significantly among pairs.

None of the tested variables was responsible for variation in fertilization rate (difference in SVL: $t = -1.56$, $p = 0.13$; ratio of SVL: $t = 1.52$, $p = 0.14$; male SVL: $t = 0.01$, $p = 0.93$). The best fit was achieved using the difference in SVL of paired toads and it only explained 8% of the variation (fig. 3). As with the previous analysis, the lowest statistically significant effect converted to R^2 would be 0.22.

Discussion

Occurrence of size-dependent and size-assortative mating

The common toad is a well-studied explosively breeding amphibian. In our experiment, as well as in few other studies (e.g., Davies and Halliday, 1977; Reading, 2001), the toads paired non-randomly with respect to size. Their mating was both size-dependent and size-assortative. Correlation of male and female SVL in pairs was significant, but the strength of this relationship was rather weak ($r = 0.33$). This

level of correlation seems, however, to be common among other studies on SAM in amphibians that have reported both significant (e.g., Howard, 1978; Berven, 1981; Gramapurohit and Radder, 2012; Vojar et al., 2015) and non-significant (Davies and Halliday, 1977; Berven, 1981; Gatz, 1981; Lee and Park, 2009) relationships. This apparent inconsistency could have resulted from small sample sizes common in studies of SAM (Vojar et al., 2015). In order to properly compare the results of correlation analyses, we strongly advise the use of power analysis (see Cohen, 1988) prior to the start of an experiment and to focus on the effect size represented by correlation coefficient. We believe that the effect size should be at least “medium”, which Cohen (1988) defines using the cut-off value of 0.3. For example, if the correlation coefficient between female and male SVL in pairs is 0.3, the difference between female SVL of 1 SD (in our case 6.08 mm) would result in corresponding change in male SVL of 0.3 SD (in our case 1.83 mm). In our opinion any significant results having correlation coefficients with lower value should be treated with care.

Effect of SAM on fertilization rate

The greatest advantage of SAM is believed to be its potential effect on fitness (Burley, 1983). Unfortunately, only few studies actually tried to measure it. This effect was not found in our study, however, and neither the difference between nor ratio of SVL in pairs resulted in a corresponding change in proportion of fertilized eggs. Even if considered as a consequence of insufficient statistical power, its effect would be very low. These findings are the opposite of those described by Bastos and Haddad (1996) in *Dendropsophus elegans*, where the ratio of male and female SVL in pairs was responsible for 85% of variability in fertilization rate. Although Ryan (1983) had found a weak correlation between the difference in body sizes of males and females of paired *Engystomops pustulosus* and fertilization success ($r = 0.30$; $n = 68$, $p < 0.05$), the effect of body size

difference in pairs had in that case explained only 9% of variability. That level was similar to that in our study (8%). The difference in significance is probably a consequence of different sample sizes between the studies (68 vs. 30 pairs), rather than different effect sizes.

Of the studies that revealed no significant relationship between SAM and fertilization success, Kruse (1981) found a negative correlation ($r = -0.30$, $n = 19$, $p > 0.2$) of fertilization success and the ratio of male and female SVL in pairs of the American toad (*Anaxyrus americanus*). In this case, the estimated power for $\alpha = 0.05$ would be 0.24. Gerhardt et al. (1987) found no significant relationship between the relative size of mates and fertilization success in their study of green treefrogs (*Hyla cinerea*), but they do not provide the precise results of their statistical analyses. Their study, like that of Kruse (1981), had a small sample size ($n = 21$). Briggs (2008) revealed no significant relationship between the ratio of SVL in pairs and fertilization success in the red-eyed leaf frog (*Agalychnis callidryas*) ($r = 0.20$, $n = 56$, $p = 0.15$) or in the black-eyed leaf frog (*Agalychnis moreletii*) ($r = 0.19$, $n = 46$, $p = 0.21$), and there was virtually no variation in fertilization success. There were only two clutches with <100% of fertilized eggs in *A. callidryas* and just one in *A. moreletii*. Fan, Lin and Ji (2013) found no significant relationship between fertilization success and ratio of mate SVL in either Asiatic toads (*Bufo gargarizans*) ($n = 65$, $p = 0.16$) or Asian common toads (*Duttaphrynus melanostictus*) ($n = 149$, $p = 0.16$).

The reports of SAM often share the weak magnitude of correlation between SVL in pairs. Considering that the effect of the size difference in pairs on fertilization success may be also very weak, there seems to be almost no benefit of SAM to this component of fitness in amphibians. Other than that it could affect fitness through mechanical constraints of amplexus (i.e. assortatively mating animals would have a higher probability of clutch deposition), there might be a benefit in a smaller chance for

displacement by a rival male. The frequency of successful displacements, however, remains unknown for most species.

Conclusions

The possible effect of SAM on fertilization success in anurans has not been studied nearly enough to draw any major inferences about its existence. The majority of existing studies, however, do not support the commonly accepted idea of maximizing fertilization success through SAM. The statistical power and the effect size in these studies are often poor and the methods used among these studies are inconsistent. We advise the use of power analysis prior to conducting an experiment and, when it is not possible to ensure an appropriate sample size, it would be more appropriate to interpret the results based upon the effect size rather than upon the test of significance. In this light, manipulative experiments involving further taxa will be needed if non-random mating in amphibians is to be properly described.

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References

- Andersson, M. (1994): Sexual Selection. Princeton University Press, Princeton, New Jersey.
- Arak, A. (1983): Male-male competition and mate choice in anuran amphibians. In: Mate Choice, p. 181-210. Bateson, P., Ed., Cambridge University Press, Cambridge.
- Arntzen, J. (1999): Sexual selection and male mate choice in the common toad, *Bufo bufo*. Ethol. Ecol. Evol. **11**: 407-414.
- Bastos, R., Haddad, C. (1996): Breeding activity of the neotropical treefrog *Hyla elegans* (Anura, Hylidae). J. Herpetol. **30**: 355-360.
- Berven, K.A. (1981): Mate choice in the wood frog, *Rana sylvatica*. Evolution **35**: 707-722.
- Bortolotti, G.R., Iko, W.M. (1992): Non-random pairing in American kestrels: mate choice versus intra-sexual competition. Anim. Behav. **44**: 811-821.

- Boyle, D.G., Boyle, B.D., Olsen, V., Morgan, J.A., Hyatt, A.D. (2004): Rapid quantitative detection of chytridiomycosis (*Batrachochytrium dendrobatidis*) in amphibian samples using real-time Taqman PCR assay. *Dis. Aquat. Organ.* **60**: 141-148.
- Brede, E.G., Beebee, T.J.C. (2006): Large variations in the ratio of effective breeding and census population sizes between two species of pond-breeding anurans. *Biol. J. Linn. Soc.* **89**: 365-372.
- Briggs, V.S. (2008): Mating patterns of red-eyed treefrogs, *Agalychnis callidryas* and *A. moreletii*. *Ethology* **114**: 489-498.
- Brown, W. (1993): The cause of size-assortative mating in the leaf beetle *Trirhabda canadensis* (Coleoptera: Chrysomelidae). *Behav. Ecol. Sociobiol.* **33**: 151-157.
- Burley, N. (1983): The meaning of assortative mating. *Ethol. Sociobiol.* **4**: 191-203.
- Cohen, J. (1988): *Statistical Power Analysis for the Behavioral Sciences*. Lawrence Erlbaum Associates, Mahwah, New Jersey.
- Crespi, B.J. (1989): Causes of assortative mating in arthropods. *Anim. Behav.* **38**: 980-1000.
- Davies, N.B., Halliday, T.R. (1977): Optimal mate selection in the toad *Bufo bufo*. *Nature* **269**: 56-58.
- Davies, N.B., Halliday, T.R. (1979): Competitive mate searching in male common toads, *Bufo bufo*. *Anim. Behav.* **27**: 1253-1267.
- Elmberg, J. (1987): Random mating in a boreal population of European common frogs *Rana temporaria*. *Ecography* **10**: 193-195.
- Emerson, S.B. (1997): Testis size variation in frogs: testing the alternatives. *Behav. Ecol. Sociobiol.* **41**: 227-235.
- Fan, X.-L., Lin, Z.-H., Ji, X. (2013): Male size does not correlate with fertilization success in two bufonid toads that show size-assortative mating. *Curr. Zool.* **59**: 740-746.
- Ferrer, M., Penteriani, V. (2003): A process of pair formation leading to assortative mating: passive age-assortative mating by habitat heterogeneity. *Anim. Behav.* **66**: 137-143.
- Fisher, R.A. (1921): On the "probable error" of a coefficient of correlation deduced from a small sample. *Metron* **1**: 3-32.
- Freeberg, T.M. (1996): Assortative mating in captive cowbirds is predicted by social experience. *Anim. Behav.* **52**: 1129-1142.
- Garland, S., Baker, A., Phillott, A.D., Skerratt, L.F. (2010): BSA reduces inhibition in a TaqMan (R) assay for the detection of *Batrachochytrium dendrobatidis*. *Dis. Aquat. Organ.* **92**: 113-116.
- Gatz, A.J. Jr. (1981): Non-random mating by size in American toads, *Bufo americanus*. *Anim. Behav.* **29**: 1004-1012.
- Gerhardt, H.C., Daniel, R.E., Perrill, S.A., Schramm, S. (1987): Mating behaviour and male mating success in the green treefrog. *Anim. Behav.* **35**: 1490-1503.
- Gosner, K.L. (1960): A simplified table for staging anuran embryos larvae with notes on identification. *Herpetologica* **16**: 183-190.
- Gramapurohit, N.P., Radder, R.S. (2012): Mating pattern, spawning behavior, and sexual size dimorphism in the tropical toad *Bufo melanostictus* (Schn.). *J. Herpetol.* **46**: 412-416.
- Hargeby, A., Erlandsson, J. (2006): Is size-assortative mating important for rapid pigment differentiation in a freshwater isopod? *J. Evol. Biol.* **19**: 1911-1919.
- Hegde, S.N., Krishna, M.S. (1997): Size-assortative mating in *Drosophila malerkotliana*. *Anim. Behav.* **54**: 419-426.
- Höglund, J., Säterberg, L. (1989): Sexual selection in common toads: correlates with age and body size. *J. Evol. Biol.* **2**: 367-372.
- Howard, R.D. (1978): The evolution of mating strategies in bullfrogs, *Rana catesbeiana*. *Evolution* **32**: 850-871.
- Hyatt, A.D., Boyle, D.G., Olsen, V., Boyle, D.B., Berger, L., Obendorf, D., Dalton, A., Kriger, K., Hero, M., Hines, H., Phillott, R., Campbell, R., Marantelli, G., Gleason, F., Colling, A. (2007): Diagnostic assays and sampling protocols for the detection of *Batrachochytrium dendrobatidis*. *Dis. Aquat. Organ.* **73**: 175-192.
- Kruse, K.C. (1981): Mating success, fertilization potential, and male body size in the American toad (*Bufo americanus*). *Herpetologica* **37**: 228-233.
- Lee, J., Park, D. (2009): Effects of body size, operational sex ratio, and age on pairing by the Asian toad, *Bufo stejnegeri*. *Zool. Stud.* **48**: 334-342.
- Lewontin, R., Kirk, D., Crow, J. (1968): Selective mating, assortative mating, and inbreeding: definitions and implications. *Biodemography Soc. Biol.* **15**: 141-143.
- Long, J.S., Ervin, L.H. (2000): Using heteroscedasticity consistent standard errors in the linear regression model. *Am. Stat.* **54**: 217-224.
- Lu, X., Chen, W., Zhang, L., Ma, X. (2010): Mechanical constraint on size-assortative pairing success in a temperate frog: an experimental approach. *Behav. Proc.* **85**: 181-184.
- Lu, X., Ma, X., Li, Y., Fan, L. (2009): Breeding behavior and mating system in relation to body size in *Rana chensiensis*, a temperate frog endemic to northern China. *J. Ethol.* **27**: 391-400.
- Marco, A., Kiesecker, J.M., Chivers, D.P., Blaustein, A.R. (1998): Sex recognition and mate choice by male western toads, *Bufo boreas*. *Anim. Behav.* **55**: 1631-1635.
- Márquez, R., Tejedo, M. (1990): Size-based mating pattern in the tree frog *Hyla arborea*. *Herpetologica* **46**: 176-182.
- McKaye, K.R. (1986): Mate choice and size assortative pairing by the cichlid fishes of Lake Jiloá, Nicaragua. *J. Fish Biol.* **29**: 135-150.
- R Development Core Team (2015): *R: a Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna.
- Reading, C.J. (2001): Non-random pairing with respect to past breeding experience in the common toad (*Bufo bufo*). *J. Zool.* **255**: 511-518.
- Rintamäki, P.T., Lundberg, A., Alatalo, R.V., Höglund, J. (1998): Assortative mating and female clutch investment in black grouse. *Anim. Behav.* **56**: 1399-1403.

- Ryan, M.J. (1983): Sexual selection and communication in a neotropical frog, *Physalaemus pustulosus*. *Evolution* **37**: 261-272.
- Shine, R., O'Connor, D., Lemaster, M.P., Mason, R.T. (2001): Pick on someone your own size: ontogenetic shifts in mate choice by male garter snakes result in size-assortative mating. *Anim. Behav.* **61**: 1133-1141.
- Vojar, J., Doležalová, J., Solský, M. (2012): A new, harmless mesocosm design for field rearing ranid embryos and determining clutch sizes. *Herpetol. Rev.* **43**: 588-590.
- Vojar, J., Chajma, P., Kopecký, O., Puš, V., Šálek, M. (2015): The effect of sex ratio on size-assortative mating in two explosively breeding anurans. *Amphibia-Reptilia* **36**: 149-154.
- Wells, K.D. (1977): The social behaviour of anuran amphibians. *Anim. Behav.* **25**: 666-693.
- Wells, K.D. (2007): *The Ecology and Behavior of Amphibians*. University of Chicago Press, Chicago.
- Yu, T.L., Lu, X. (2010): Mating patterns of Minshan's toad (*Bufo minshanicus*) from three populations along an altitudinal gradient. *Zool. Sci.* **27**: 856-860.
- Zar, J.H. (1984): *Biostatistical Analysis*, 2nd Edition. Prentice-Hall, Englewood Cliffs.

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