

## Shelter use assessment and shelter enrichment in captive bred common toads (*Bufo bufo*, Linnaeus 1758)

Octavian CRAIOVEANU<sup>1</sup>, Cristina CRAIOVEANU<sup>1</sup>, Irina COSMA<sup>2</sup>,  
Ioan GHIRA<sup>1</sup> and Vioara MIREȘAN<sup>2</sup>

1. Babeș-Bolyai University Cluj-Napoca, Clinicilor str. 5-7, 400006

2. University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Calea Mănăstur 3-5, Cluj-Napoca 400372

\*Corresponding author, C. Craioveanu, E-mail: [cristinacraioveanu@gmail.com](mailto:cristinacraioveanu@gmail.com)

Received: 23. June 2016 / Accepted: 02. January 2017 / Available online: 09. February 2017 / Printed: December 2017

**Abstract.** The decline of amphibian populations has promoted *ex situ* conservation actions as one of the tools used to address this problem. Environmental enrichment techniques used in combination with the *ex situ* conservation protocols can increase the survival rate and the overall success of reintroduction programs. In this study we assessed the preference of post metamorphic *Bufo bufo* juveniles for seven different shelter types and their ecologically relevant characteristics in controlled laboratory conditions. We found that shelters commonly used in other studies (e.g. plastic cup, pvc pipe, ceramic clay pipe and clay tile) on frogs and toads were not suitable for *B. bufo*. In our experiment, toadlets presented a higher interest in shelters that provided the widest angle of vision, direct contact with moist substrate and green colour. Our results suggest that designing captive enclosures should always consider providing shelters that meet the species-specific ecological needs.

**Key words:** behaviour, ex-situ conservation, shelter preference.

In the past three decades, declines in amphibian populations have been reported from all over the world. Reasons for their decline are multiple, including infectious diseases (Daszak et al. 2003), climate change, over-collection, habitat loss and degradation (Stuart et al. 2004, Beebee & Griffiths 2005) and their synergistic effects (Wake 1998, Stuart et al. 2004). It is estimated that almost one-third of the 7,571 amphibian species are on the brink of extinction and represent the forefront of the sixth mass extinction (Barnosky et al. 2011, Frost 2016). The decline of amphibian populations represents a serious challenge for the conservation community (Dawson et al. 2015). Through the practice of reintroduction (translocation from another wild population or captive breeding and release), *ex situ* conservation emerges as an important way to address this issue (Gascon et al. 2007, Gagliardo et al. 2008).

Currently there are no established species-specific husbandry protocols for the large majority of amphibian species (Pough 2007). Enclosure design based on enrichment studies (Michaels et al. 2014a) may increase the survival rate in captive breeding programs. Experimenting with environmental enrichment techniques may help conservationists understand the characteristics of a good quality shelter. Shelter use confers a number of advantages to post metamorphic anurans including refuge from predators (Schwarzkopf & Alford

1996, Sih 1997), increased efficiency of prey ambush and ecophysiological benefits (e.g. thermoregulation and osmoregulation) provided by suitable microclimate (Walsh & Downie 2005). Furthermore, the use of shelter can reduce heat (e.g. desiccation, concentration of electrolytes, Shoemaker 1964) and cold-induced physiological stress (e.g. immunosuppression, Carey 2001) (Hoffman & Katz 1989). These benefits have positive impact on the welfare and overall fitness of the animals (Michaels et al. 2014a, b).

In this study we examined the shelter preference of the common toad (*Bufo bufo*, Linnaeus, 1758) using seven different types of shelter, in laboratory conditions. The aim of the study was to document optimal shelter characteristics for toad husbandry and future enrichment experiments. In order to assess the quality of different shelters, we selected the most common shelter types used in the literature complemented with new types of shelters developed in the context of this study. We tested for overall preference for the shelters. Also, considering the ambush feeding strategy and the lack of tracking eye movement in toads (Ewert, 1980), we tested their preference for the field of view that each studied shelter provides (narrow, intermediate, wide). Substrate moisture preference was also tested, since terrestrial and semi-terrestrial amphibians absorb water through osmosis across their ventral skin through the water

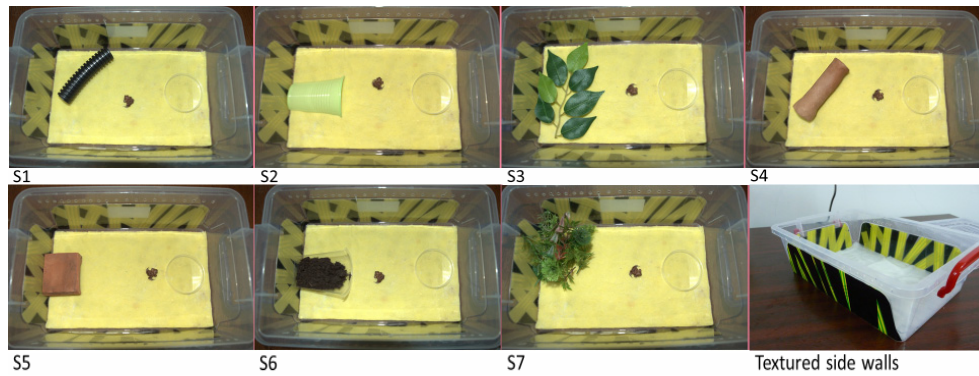


Figure 1. Images with the 7 shelter types used in the experiment (from left to right S1-S4 and S5-S7) and image depicting the texturing of side walls of the enclosures (textured side walls).

absorption response (Hillyard et al. 1998).

To perform our study we used the common toad (*Bufo bufo*) as model species. The common toad is not currently endangered (IUCN Least Concern category) and is not part of any *ex situ* conservation program. However, knowledge of their shelter preference in laboratory conditions could have implications for captive breeding and management programs of species with similar ecology.

**Field collection and tadpole raising.** Two pairs of common toads were collected from a pond near Căzănești (Hunedoara County, Romania, Lat: 46° 7' 34.65" N, Long: 22° 30' 3.54" E). The toads reproduced in captivity and were released back into their native environment afterwards. The two parent toad couples were kept in two separate aquariums in 10 cm deep dechlorinated tap water. They were not fed until the egg clutches were laid and afterwards they were released in the location where they were captured. When the metamorphosis of the larvae was completed, five toadlets were randomly selected from each spawn and the other toadlets were released. The experiment was started with 10 toadlets.

**Holding conditions.** When the metamorphosis was completed, toadlets were housed communally for one month. After this period each toadlet was individually separated in experimental enclosures consisting of specially designed transparent plastic containers (27x17x8 cm). For the accuracy of the visual function, three side-walls of these containers were textured with random patterns of yellow, black, green and opaque colours and one wall was kept transparent (Ewert 2004) (Fig.1). As substrate we used a washcloth material with good water retention capacity. Water was held in a 55mm Petri dish on the left side of the enclosure and the shelter was provided on the right side of the enclosure. The room temperature was set to 20° C. All containers were held in a 4 x 3 m room. Lighting was provided by a (2 x 18 W) fluorescent light tube, on a 15 hours light - 9 hours dark cycle. Night

time observations were made using infra-red light. Each container was cleaned once a week and dechlorinated tap water was used to maintain the substrate moisture.

Toads were fed *ad libitum* on pinhead crickets (*Acheta domestica*), that were gut-loaded with a mix of cat food, laboratory mouse food and fish pellets. Crickets were dusted before every feeding with TerraVit powder for additional minerals and vitamins.

**Experimental design.** The experiment was conducted during seven one-week trials. Seven types of shelters were provided (Table 1) representing one shelter type/week. We tested the most commonly used shelter types for frogs and toads in laboratory conditions: pvc pipe (S1) (Ewert et al. 2004, Wilson & Krause 2012, Pough 2007), plastic cup (S2) (Joke, Raoul, Matthysen, 2012, Cramp et al. 2014), ceramic clay pipe (S4) (Ewert et al. 2004) and clay tile (S5) (Gonzalo et al. 2008); and we also used shelter types not mentioned in the literature: artificial forest plant branch (S3), artificial *Ceratophyllum* plant pile (S7) and potting soil (S6) (Fig. 1.). The pvc pipe and the clay pipe were both considered dry shelters because, when in shelter, the toadlet would not come in contact with the wet washcloth that lined the floor of the enclosure. The other 5 shelter types were considered wet because, when in shelter the toadlet would come in contact with the wet washcloth beneath. When in the pvc pipe and ceramic pipe toadlets were considered to have a narrow angle of vision, when in the plastic cup an intermediate angle of vision and when under the clay tile, artificial forest plant branch, artificial *Ceratophyllum* plant pile and potting soil, had a wide angle of vision. Because during the experiment three toadlets died, only the first refuge preference trial was conducted with ten toadlets, the next two with eight toadlets and the following four trials with just seven toadlets. In total we conducted 53 toad-shelter trials.

With each shelter type, the animals were allowed a 48 hours acclimatisation period. Toadlets were kept on inverted light-dark cycles: from 17-08 light cycle (15 hours), from 08-17 dark cycle (9 hours). Each individual was observed six times (at 09.00, 13.00, 16.00, 18.00, 00.00,

Table 1. Shelter types presented in chronological order (the succession of shelters tested).

No.	Shelter type	Code
1	Black, corrugated, pvc pipe 10cm/25mm,	S1
2	Green, 1/3 disposable, plastic cup 200ml,	S2
3	Artificial forest plant branch with 7 leaves, Exoterra forest plant	S3
4	Clay pipe 10cm/25mm	S4
5	Clay tile 5cm/6cm	S5
6	Potting soil in 200ml disposable cup cut in half	S6
7	Artificial <i>Ceratophyllum</i> pile, Resun hornwort	S7

07.00 hours), three times during light periods (18.00, 00.00, 07.00 hours) and three times during dark periods (09.00, 13.00, 16.00 hours), for three days. During each observation, we recorded the position of the animal according to Table 2.

**Data analysis.** Individual responses were compared to assess patterns of individual preferences for shelters or positions in the enclosure. We tested first to see whether for each shelter type there were large variations between individual responses to avoid bias when testing for overall preferences of toads for different shelters. Because we found no large variation in the response between individuals we tested overall preferences of toads for different shelters and enclosure use as follows. For each shelter we assessed the proportion of observations (time) in which the toads were using it (position 1 – Table 2), i.e. the number of times they were observed in shelters divided by the total number of observations. We averaged the proportions over all individuals, to obtain an average proportion of time spent in shelter for each trial. We compared distributions of proportions of time spent by toads in the seven types of shelter tested with the non-parametric Kruskal-Wallis test followed by Mann-Whitney pairwise comparisons, because data was not normally distributed. In order to check whether there is a relationship between distance from shelter and time spent in different positions (Table 2), in different shelter types, we analysed the data with the help of Spearman rank correlation. First, analyses were performed on all positions and then positions were removed, keeping for further analysis always the highest value of Rho. To check whether the angle of vision and substrate moisture influence shelter use we attributed to each of the two variables a rank: angle of vision: narrow = 1, intermediate = 2, wide = 3, moisture: dry = 1, wet = 2. Subsequently we compared proportion of time toads were observed in wide versus intermediate and narrow angle of vision (when in shelter), and moist versus dry shelters respectively. Non-parametric methods (i.e. Mann-Whitney U-test, Kruskal-Wallis) were used for the comparisons, due to the non-normal distribution of data and small sample sizes. Using the same nonparametric methods, we tested if there are differences in shelter use between diurnal and nocturnal conditions.

Data was analysed with the program Past 2.09 (Hammer et al. 2001).

Table 2. Position codes.

code	Position related to the shelter
1	In shelter
2	Close to shelter, maximally one body length away
3	In the shelter half of the enclosure, excluding position 2
4	In the half of the enclosure without shelter
5	In water

We found no differences between individual responses to each shelter type (data not shown).

All toads showed a preference for the artificial forest plant branch (S3) and artificial *Ceratophyllum* pile (S7). More than 80% of times they were spotted inside these two shelters. Shelter use response (proportion of time observed in the first position of five possibilities) pooled over all individuals was significantly different in different shelter types (Kruskal-Wallis test,  $H_c=33.60$ ,  $p=0.001$ ). Mann-Whitney pairwise comparisons showed that preference for artificial forest plant branch (S3) was significantly different than for black pipe (S1) (Bonferroni corrected  $p = 0.01$ ), clay tile (S5) (Bonferroni corrected  $p = 0.047$ ) and potting soil (S6) (Bonferroni corrected  $p = 0.039$ ) (Fig. 2). Preference for artificial *Ceratophyllum* pile (S7) differed significantly from that for black pipe (S1) (Bonferroni corrected  $p = 0.027$ ).

When considering the distance from the shelter and the time spent by toads in the respective positions, we found a strong negative relationship (i.e. less time spent in positions further from the shelter) only in the situations where shelters were preferred (S3 and S7 – Table 3 marked with '\*'). Other situations showed a similar negative relationship only after removing from the analysis the 1<sup>st</sup> position (in shelter) (Table 3 marked with '\*\*').

We found that toads spent a significantly different proportion of time in shelters that offered a wider angle of view compared to those with intermediate and narrow angles (Kruskal-Wallis test:  $H_c = 13.41$ ,  $p = 0.001$ , Bonferroni corrected Mann-Whitney pairwise comparisons: wide vs. intermediate  $p = 0.011$ , wide vs. narrow  $p = 0.003$ ) (Fig. 3).

Toads spent significantly different amounts of time in shelters with substrate moisture than in dry ones (Mann-Whitney U-test: N moist = 8, N dry = 10,  $z = -2.713$ ,  $U_b = 9$ ,  $p = 0.007$ , fig. 4).

Shelter use did not differ significantly between day and night in any shelter type used (Mann-Whitney U-test: N day = 8, N night = 8, Median day = 0.889, Median night = 0.944,  $z = -0.479$ ,  $U_b =$

Table 3. Results of the correlation analysis between position (distance from shelter) and proportion of time spent in the different shelter types.

Type of shelter (code)	Correlation analysis: position (distance from shelter) vs. proportion of time spent	Rho	p
S1	all positions included	-0.380	0.016
	position 1 removed**	-0.759	0.001
	positions 1 and 5 removed	-0.710	0.001
	positions 1, 3 and 5 removed	-0.707	0.005
S2	all positions included	-0.380	0.014
	position 2 removed	-0.557	0.001
	positions 1 and 2 removed	-0.804	0.001
	positions 1, 2 and 4 removed	-0.907	0.001
S3	all positions included*	-0.734	0.001
	position 4 removed	-0.828	0.001
	positions 3 and 4 removed	-0.894	0.001
	positions 2, 3 and 4 removed	-0.907	0.001
S4	all positions included	-0.421	0.012
	position 1 removed**	-0.731	0.001
	positions 1 and 3 removed	-0.804	0.001
	positions 1, 3 and 4 removed	-0.928	0.001
S5	all positions included	-0.456	0.006
	position 1 removed**	-0.950	0.001
	positions 1 and 4 removed	-0.955	0.001
	positions 1, 2 (3) and 4 removed	-0.933	0.001
S6	all positions included	-0.167	0.339
	position 1 removed**	-0.671	0.001
	positions 1 and 2 removed	-0.789	0.001
	positions 1, 2 and 4 removed	-0.928	0.001
S7	all positions included*	-0.799	0.001
	position 3 removed	-0.849	0.001
	positions 3 and 4 (5) removed	-0.894	0.001
	positions 2, 3 and 4 removed	-0.932	0.001

\*Expected relationship between distance and time spent in shelter - for preferred shelters.

\*\*Expected relationship between distance and time spent in shelter after removing first position.

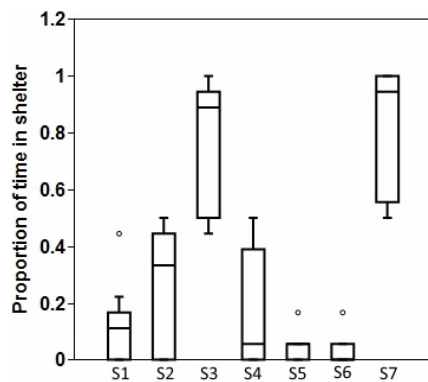


Figure 2. Comparisons between proportions of time the different shelter types were used by all toads. Box plots represent 25-75 percent quartiles (boxes), median (line inside the box) and minimal and maximal values (whiskers). Circles represent outliers. Legend: S1 = black pvc pipe, S2 = green plastic cup, S3 = artificial forest plant branch, S4 = clay pipe, S5 = clay tile, S6 = potting soil, S7 = artificial *Ceratophyllum* plant.

27,  $p = 0.632$ ).

The aim of this study was to determine the shelter preference of *B. bufo* toadlets in laboratory conditions. Our results show that toads prefer shelters that mimic natural conditions, artificial forest plant branch (S3) and artificial *Ceratophyllum* pile (S7) (Fig. 1, 2). None of these shelter types were previously mentioned in the literature for other frog and toad species. In the wild, *B. bufo* occurs in areas with multiple refuge options. Angle of vision and substrate moisture play important roles in the choice of a shelter.

Toads seem to have good depth and colour perception adapted to vision under conditions of dim light (Stebbins & Cohen 1997). As we hypothesized, angle of vision seems to be important for the toadlets' ambush feeding strategy and the lack of tracking eye movement (Ewert 1980). A wide angle of vision also provides more efficient

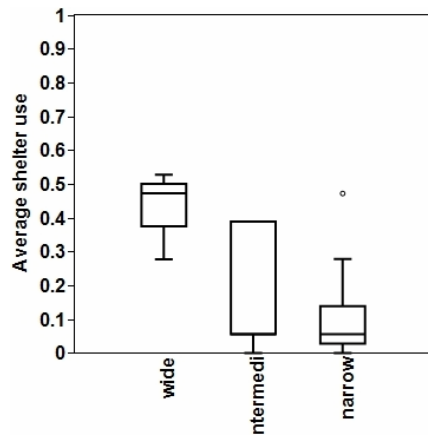


Figure 3. Average proportion of time (Median wide = 0.479, Median intermediate = 0.056, Median narrow = 0.056) spent by toads in wide angle, intermediate angle and narrow angle of vision shelters. Box plots represent 25-75 percent quartiles (boxes), median (line inside the box), minimal and maximal values (whiskers) and dots represent outliers. Circles represent outliers.

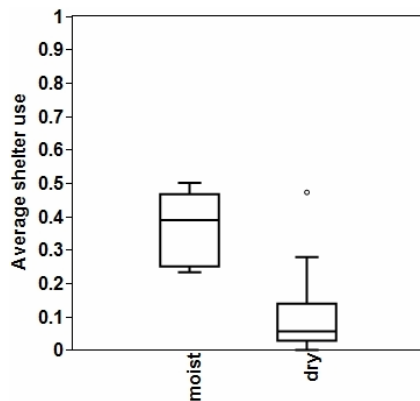


Figure 4. Average proportion of time (Median moist = 0.395, Median dry = 0.084) spent by toads in moist and dry shelters. Box plots represent 25-75 percent quartiles (boxes), median (line inside the box), minimal and maximal values (whiskers) and dots represent outliers. Circles represent outliers.

foraging possibilities with minimal effort; therefore activity levels were low when preferred shelters were used.

The humidity benefits of a good shelter were previously mentioned for terrestrial amphibians, (Hillyard et al. 1998), but for toadlets this could be especially important because of their higher surface/volume ratio (Walsh & Downie 2005). Furthermore, we observed during the experiment that

colour of shelters might play an important role in shelter choice. However, since our experiment did not consider this factor from the beginning, we cannot prove it by statistical analysis.

The results of the correlation analysis show a strong negative correlation between distance to shelter and time spent by *B. bufo* toadlets in the case of preferred shelters. The toadlets seem to choose to hide in shelters in order to minimize exposure and the process is enhanced when preferred refuge is available. However, when shelter quality was low, toadlets tend to spend more time in the open. The choice of *B. bufo* to use a certain type of refuge, when available, suggest that animals provisioned with this type of shelter may experience less stress, so this form of environmental enrichment is to be encouraged.

Field studies suggested that small, just metamorphosed toads are diurnal, unlike adults which are nocturnal (Eibl-Eibesfeldt 1967, Taigen & Pough 1981). In this experiment however, we found no differences between the patterns of shelter use during dark and light periods. When a shelter type was accepted and used, the toadlets spent more than 80% of their time inside. This suggests that shelter might be especially important in this life stage for *B. bufo*.

Overall, the results of this experiment show that designing capture enclosures with good quality shelters is particularly important for *B. bufo* in laboratory conditions, especially in its early life stages and may influence the long-term fitness of captive populations and the chances of success in the case of reintroduction attempts. We emphasise the need for using shelters which mimic as much as possible the natural conditions. Our findings might be applicable as starting protocols for captive breeding of other toad species with similar ecology until more species-specific data is available.

**Acknowledgements.** We thank Prof. Adalbert Balogh for his valuable critiques and for many constructive discussions on the statistical analyses of this paper. We are grateful to Martyn Davies who voluntarily offered his friendly help with English language corrections.

#### References

- Barnosky, A.D., Matzke, N., Susumu, T., Wogan, O.U.G., Swartz, B., Quental, T.B., Marshall, C., McGuire, J.L., Lindsey, E.L.,

- Maguire, K.C., Mersey, B., Ferrer, E.A. (2011): Has the Earth's sixth mass extinction already arrived? *Nature* 147: 51-57.
- Beebee, T.J.C., Griffiths, R.A. (2005): The amphibian decline crisis: a watershed for conservation biology? *Biological Conservation* 125: 271-285.
- Carey, C. (2002): Hypothesis concerning the disappearance of Boreal Toads from the mountains of Colorado. *Conservation Biology* 7(2): 355-362.
- Cramp, R.L., McPhee, R.K., Meyer, E. A., Ohmer, M.E., Franklin, C. E. (2014): First line of defence: the role of sloughing in the regulation of cutaneous microbes in frogs. *Conservation Physiology* 2(1): cou012.
- Daszak, P., Cunningham, A.A., Hyatt, A.D. (2003): Infectious disease and amphibian population declines. *Diversity and Distributions* 9: 141-150.
- Dawson, J., Patel, F., Griffiths, R. A., Young, R.P. (2015): Assessing the global zoo response to the amphibian crisis through 20-year trends in captive collections. *Conservation Biology* 30(1):82-91.
- Eibl-Eibesfeldt, I. (1967): Nahrungserwerb und Beuteschema der Erdkröte. *Behavior* 4:1-35.
- Ewert, J.P. (1980): Neuroethology-An Introduction to the Neurophysiological Fundamentals of Behavior. Springer-Verlag.
- Ewert, J.P., Cooper, J.E., Langton, T., Matz, G., Reilly, K., Schwantje, H. (2004): Background information On the species-specific proposals for amphibians Presented by the Expert Group on Amphibians and Reptiles In Working party for the preparation of the fourth multilateral consultation of parties to the European convention for the protection of vertebrate animals used for experimental and other scientific (ETS 123) 8th Meeting of the Working Party Strasbourg, 22-24 September 2004.
- Frost, D.R. (2016): Amphibian species of the world: an Online Reference. Version 6.0 (Date of access). American Museum of Natural History, New York, USA.
- Gagliardo, R., Crump, P., Griffith, E., Mendelson, J., Ross, H., Zippel, K. (2008): The principles of rapid response for amphibian conservation, using the programs in Panama as an example. *International Zoo Yearbook* 42(1): 125-135.
- Gascon, C., Collins, J.P., Moore, R.D., Church, D.R., McKay, J.E., Mendelson III, J. (2007): Amphibian conservation action plan. Proceedings: IUCN/SSC Amphibian Conservation Summit 2005; The World Conservation Union (IUCN), Gland, Switzerland.
- Hillyard, S.D., Hoff, K.S., Propper, C. (1998): The water absorption response: a behavioral assay for physiological processes in terrestrial amphibians. *Physiological Zoology* 71(2):127-138.
- Hoffman, J., Katz, U. (1989): The ecological significance of burrowing behavior in the toad (*Bufo viridis*). *Oecologia* 81: 510-513.
- Joke, M., Van Damme, R., Matthysen, E. (2013): Individual and among-population variation in dispersal-related traits in Natterjack toads. *Behavioral ecology* 24(2): 521-531.
- Michaels, C.J., Antwis, R.E., Preziosi, R.F. (2014a): Impact of Plant Cover on Fitness and Behavioural Traits of Captive Red-Eyed Tree Frogs (*Agalychnis callidryas*). *PLoS ONE* 9(4): e95207.
- Michaels, C.J., Gini, B., Preziosi, R.F. (2014b): The importance of natural history and species-specific approaches in amphibian *ex-situ* conservation. *Herpetological Journal* 24: 135-145.
- Parris, M.J. (1998): Terrestrial burrowing ecology of newly metamorphosed frogs (*Rana pipiens* complex). *Canadian Journal of Zoology* 76: 2124-2129.
- Pough, F.H. (2007): Amphibian Biology and Husbandry. *ILAR journal* 48 (3): 203-213.
- Schwarzkopf, L., Alford, R.A. (1996): Desiccation and shelter-site use in a tropical amphibian: comparing toads with physical models. *Functional Ecology* 10: 193-200.
- Seebacher, F., Alford, R. A. (2002): Shelter microhabitats determine body temperature and dehydration rates of a terrestrial amphibian (*Bufo marinus*). *Journal of Herpetology* 36: 69-75.
- Shoemaker, V.H. (1964): The effects of dehydration on electrolyte concentrations in a toad, *Bufo marinus*. *Comparative Biochemistry and Physiology* 13: 261-271.
- Sih, A. (1997): To hide or not to hide? Refuge use in a fluctuating environment. *Trends in Ecology and Evolution* 12: 375-376.
- Stebbins, R.C., Cohen, N. W. (1997): A Natural History of Amphibians. ISBN: 9780691102511, Princeton University Press.
- Stuart, S.N., Chanson, J.S., Cox, N.A., Young, B.E., Rodrigues, A.S.L., Fischman, D.L., Waller, R.W. (2004): Status and trends of amphibian declines and extinctions worldwide. *Science* 306: 1783-1786.
- Taigen, T.L., Pough F.H. (1981): Activity Metabolism of the Toad (*Bufo americanus*): Ecological Consequences of Ontogenetic Change. *Journal of comparative physiology* 144(2): 247-252.
- Wake, D.B. (1998): Action on amphibians. *Trends in Ecology & Evolution* 13: 379-380.
- Walsh, P.T., Downie, J.R. (2005): The effects of shelter availability and substrate quality on the behavior and post-metamorphic growth in three species of anurans: implications for captive breeding. *Herpetological journal* 15: 245-255.