ALYTES
INTERNATIONAL JOURNAL OF BATRACHOLOGY
ISSCA
RESEARCH ARTICLE
2015 | VOLUME 32 | PAGES 7-15

Effects of three diets on development of *Mantidactylus betsileanus* larvae in captivity

Jeanne Soamiarimampionona[1], Sidonie Samina Sam[1], Rainer Dolch[1], Katy Klymus[2], Falitiana Rabemananjara[3], Eric Robsomanitrandrasana[4], Justin Claude Rakotoarisoa[1], Devin Edmonds[1]*

[1] Association Mitsinjo, Andasibe, Madagascar
[2] University of Toledo, Lake Erie Center, Oregon OH, U.S.A.
[3] Amphibian Specialist Group and the Société pour la Conservation des Amphibiens de Madagascar (SCAM), Ambohimanga, Bongatsara, Madagascar
[4] La Direction Générale des Forêts, Nanisana, Antananarivo, Madagascar

Conservation breeding programmes are increasingly needed for amphibians given the ongoing amphibian extinction crisis, yet key aspects of husbandry such as larval diet remain understudied and in many cases completely unknown. In Madagascar, enacting such programmes can also be challenging due to the unavailability of diets designed specifically for tadpoles. We tested three diets locally available in Madagascar — mustard greens, spirulina algae, and atyid shrimp — on the larvae of *Mantidactylus betsileanus* and recorded their growth and development. Tadpoles fed mustard greens took longer to develop and completed metamorphosis at a smaller size. No difference was found in the survival of tadpoles between treatments. These results suggest that mustard greens are a poor food source for rearing *M. betsileanus* and similar species. Instead, spirulina and atyid shrimp should be used, although other alternatives such as commercially manufactured tadpole and fish foods might yield even better results.

INTRODUCTION

Captive breeding programmes can be effective in combating amphibian extinctions and population declines, especially in light of the threat presented by emerging infectious diseases, such as chytridiomycosis (Gagliardo *et al*., 2008; Griffiths & Pavajeau, 2008; Tapley *et al*., in press).To help offset population declines, offspring produced in captivity can be used to supplement declining wild populations, selected for increased disease resistance or reproductive capacity, or reintroduced at created habitat in disease-free locations (Scheele *et al*., 2014). In order to have the capacity to implement these types of programs, it is essential to have an understanding of the natural history of the target species and ideally to have already on hand a protocol developed for best captive care practices (Michaels *et al*., 2014).

The nutritional requirements of amphibians in captivity are one of the most important aspects of captive management, yet they remain understudied compared to other taxa such as reptiles or fish (McWilliams, 2008; Pessier, 2010). The diet of larvae in particular is important because it has significant effects on their survival, growth, and development, the relationship of which has been studied in a number of anuran species (Steinwascher & Travis, 1983; Leips & Travis, 1994; Carmona-Osalde, 1996; Álvarez & Nicieza, 2002). Special care needs to be taken to ensure that the food offered to larvae produces healthy adult animals, in particular when long-term sustained viability of a captive population is needed (McCallum & Trauth, 2002).

Understanding the diet of a species in nature can help guide captive rearing techniques (Pramuk & Gagliardo, 2012), however, the natural history and feeding habits of the majority of amphibian species in their

Received 01 May 2015
Accepted 04 August 2015
Published Online 05 October 2015
*Corresponding author devin@amphibiancare.com

larval stage remain largely unknown (Altig *et al*., 2007; Wells, 2007). Many questions remain regarding the optimal diet for most species in captivity, providing an opportunity to test hypotheses about different foods and their effects on development of offspring. What constitutes the optimal diet may vary depending on the goals of the breeding programme, but in general it is advantageous for tadpoles to complete metamorphosis at a large size and in a short time to increase their overall fitness (Martins *et al*., 2013). It's also important to consider survivorship post metamorphosis and avoid unintentional captive selection pressures and genetic bottlenecks, especially if the purpose of the captive population is to supply stock for reintroductions (Christie *et al*., 2012). Determining the best food takes both of these points into consideration, alongside the availability of different food types and costs to the breeding programme.

We developed a facility in Andasibe, Madagascar in 2011 to enact captive breeding programmes as part of the national amphibian conservation strategy known as the Sahonagasy Action Plan (Andreone & Randriamahazo, 2008; Edmonds *et al*., 2012). One of the goal's of the project is to determine the captive care and husbandry requirements for the numerous Malagasy amphibian species that have yet to be managed in captivity (see García *et al*., 2008) so that if population declines or extinctions are detected survival assurance colonies can rapidly be established. This is especially important in light of the recent news that the amphibian chytrid fungus *Batrachochytrium dendrobatidis* is widespread on the island (Bletz *et al*., 2015). Because of its somewhat remote location, the breeding facility relies on locally available materials as much as possible to avoid being dependent on imported supplies since commercial tadpole diets are not available in Madagascar and aquarium fish foods can only occasionally be found for sale, and even then are often already expired or are sold at exaggerated prices. As a result, we reviewed products available locally in and around Andasibe that could be used to feed tadpoles produced at the breeding facility.

Our focal species for the study, the Betsileo Madagascar Frog (*Mantidactylus betsileanus*), is known from the areas around Andasibe and Ranomafana in eastern Madagascar. It is semi-aquatic and found in the vicinity of rainforests, where it breeds in slow-moving streams, swampy areas, and associated shallow water bodies (Glaw & Vences, 2007; Vences & Nussbaum, 2008). The larvae of *M. betsileanus* have been raised in captivity a few times (Arnoult & Razarihelisoa, 1967; Blommers-Schlösser, 1979; Scheld *et al*., 2013), with tadpoles in the latter study being fed a diet of fish flake and algae. It is not clear whether the two earliest reports were actually *M. betsileanus* or one of the numerous similar cryptic species only recently recognized through DNA-barcoding (Vieites *et al*., 2009; Perl *et al*., 2014), nevertheless there is still much to be learned about this species' optimal requirements in captivity.

In this article we describe the results of an experiment to determine the effects of three diets on the survival and development of *M. betsileanus* larvae in captivity. Based on qualitative observations raising larvae on mixed diets previously, we predicted that a diet of dried atyid shrimp would result in higher survivorship, faster growth, and larger size at metamorphosis compared to diets of spirulina algae or mustard greens. We conclude by discussing the importance of diet during the larval stage of development in anurans and the significance of captive husbandry and zoo-based research considering the ongoing amphibian extinction crisis.

MATERIALS AND METHODS

Captive rearing methods and experimental design

Two egg masses from different breeding groups were used to source larvae for the study. Both egg masses were located on a substrate of gravel on land near water, one concealed under a segment of PVC plastic pipe and the other under half of a coconut shell. The first egg clutch was discovered on 2 January 2013 and contained 83

Table 1. Summary of breeding events that supplied tadpoles for the study.

Breeding group	Location of eggs in terrarium	Date eggs found	Date larvae emerged from eggs	# Eggs	# Tadpoles	# Tadpoles in experiment
MABE-B	On gravel under half of a coconut shell	02-Jan-2013	08-Jan-2013	83	8	5
MABE-A1	On gravel under PVC plastic pipe segment	16-Jan-2013	16-Jan-2013	44	44	39

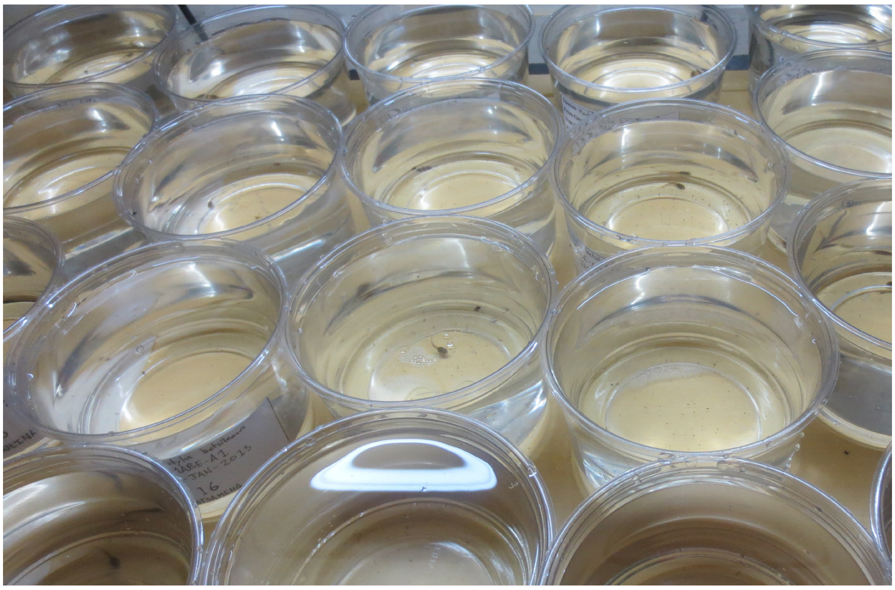

Figure 1. Tadpoles housed individually in 16 oz. plastic cups.

eggs, only 8 of which were fertile. The 8 larvae emerged from the egg mass in a separate container outside the terrarium one week later. The second egg mass was found on 16 January 2013 and contained 44 well developed larvae which were starting to hatch the day they were found (tab. 1). On 24 January 2013 we moved all surviving tadpoles to individual 16 oz. (473 ml) plastic cups filled with 2-3 cm (~60-120 ml) of water (fig. 1). We assigned separate identification numbers to each tadpole, with a B preceding the number to indicate the individuals from the first egg clutch that was largely infertile. Tadpoles were later moved on 28 March 2013 to larger aquariums to prevent escapes, which had by this point caused the loss of 7 individuals. The new aquariums measured 45 x 25 x 25 cm and were filled with about 12 cm (~13.5 l) of water.

Water for the study was sourced from the tap and was unfiltered, originating from a stream ~ 3km away in Andasibe National Park. We performed complete water changes daily while tadpoles were in the 16 oz. (473 ml) cups, and complete water changes weekly once moved to aquariums. Once per week each tadpole was examined closely with a magnifying glass and their Gosner stage (Gosner, 1960) recorded. During this time we also recorded the weekly minimum and maximum water temperature as well as pH, ammonia, nitrite, and nitrate of our water source using colormetric aquarium water tests. Water temperature was allowed to fluctuate naturally with the ambient room temperature, which varied seasonally to as low as 12.8°C in June and as high as 27.5°C in February. Average water temperature over the entire experiment was recorded as 16.5°C (weekly low) and 26.5°C (weekly high).

Table 2. Summary of diet treatments.

Treatment	Diet	No. tadpoles that survived through metamorphosis	No. tadpoles that died during metamorphosis
A	Spirulina	7	2
B	Atyid shrimp	12	1
C	Mustard greens	13	1

Three different diet treatments were used to test our hypothesis that a diet of shrimp would result in higher survivorship, faster development and larger frogs (tab. 2). Treatment A (14 tadpoles) were fed powder spirulina algae, treatment B (15 tadpoles) were fed ground dried atyid shrimp, and treatment C (15 tadpoles) were fed sun-dried ground mustard greens. During the course of the study some of the tadpoles escaped their enclosure and were lost. These individuals were not included in any measurements, thus the total number of individuals per treatment were A (9), B (13), C (14). All foods were sourced locally from markets near Andasibe, the shrimp commonly known in Malagasy as *patsamena* and greens *ananamanitra*. Each tadpole was fed a small pinch of the diet (about 0.05-0.10 g) daily, as much as they could eat without polluting the water.

When tadpoles reached Gosner stages 41-42, we moved them from aquariums to 32 oz. (946 ml) ventilated plastic containers with less than 1 cm (~20-40 ml) of water and several dried leaves as surfaces to help prevent drowning. Upon completing metamorphosis, individuals were weighed (0.01 g) with an Ohaus TAJ402 digital scale and their snout to vent length measured with a plastic calliper measured to the nearest millimetre.

Statistical analysis

We tested for differences in days to metamorphosis, length at metamorphosis and weight at metamorphosis among the three feeding treatments (tab. 3). These traits were chosen as they likely relate to an individual's fitness. For instance, the sooner they complete metamorphosis, the sooner the frog can reproduce, while completing metamorphosis at a larger size (greater mass and length) might indicate an increased chance to survive compared to smaller individuals (Formanowicz & Brodie, 1982; Werner, 1986; Newman, 1998; Altwegg & Reyer 2007).

We first ran Shaprio-Wilk tests to assess normality of each dataset. As the three datasets (days to metamorphosis, length at metamorphosis and weight at metamorphosis) were found to not follow a normal distribution, non-parametric Kruskal-Wallis tests were used to detect differences among the three feeding treatments. Finally, post-hoc pairwise Mann-Whitney U tests with Bonferroni correction were run to assess which treatments were statistically significant ($p < 0.05$).

Table 3. Days to metamorphosis, length at metamorphosis and weight at metamorphosis.

ID	Treatment	Days to metamorphosis	SVL at metamorphosis (mm)	Weight at metamorphosis (g)
B1	Spirulina	119	12.0	0.16
4	Spirulina	112	11.0	0.17
5	Spirulina	98	12.0	0.10
6	Spirulina	105	12.0	0.22
8	Spirulina	112	12.0	0.21
9	Spirulina	105	12.5	0.14
10	Spirulina	105	12.0	0.20
15	Shrimp	84	11.0	0.09
16	Shrimp	98	12.0	0.22
19	Shrimp	91	11.0	0.16
B20	Shrimp	119	12.0	0.12
B21	Shrimp	147	12.0	0.15
22	Shrimp	98	12.0	0.14
23	Shrimp	98	13.0	0.22
24	Shrimp	119	18.0	0.18
25	Shrimp	98	12.0	0.22
26	Shrimp	91	12.0	0.14
27	Shrimp	98	12.0	0.16
28	Shrimp	98	12.0	0.20
29	Greens	189	9.0	0.08
30	Greens	147	11.0	0.09
32	Greens	133	9.0	0.09
33	Greens	209	10.0	0.19
34	Greens	157	12.0	0.12
35	Greens	164	12.0	0.10
36	Greens	171	11.0	0.12
37	Greens	168	9.0	0.09
38	Greens	161	10.0	0.10
39	Greens	140	10.0	0.10
41	Greens	280	12.0	0.12
42	Greens	154	11.0	0.19
43	Greens	154	11.0	0.15

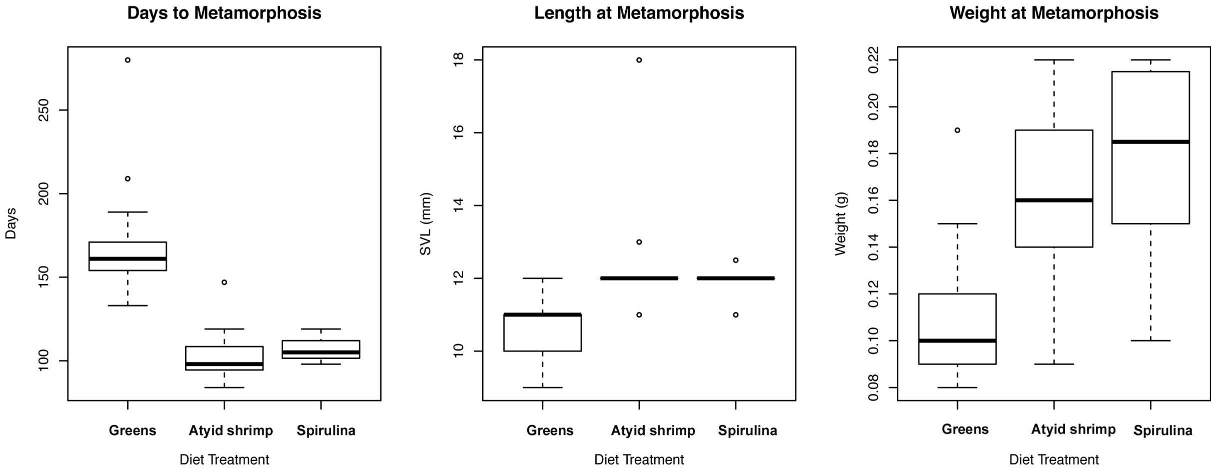

Figure 2. Box plots showing days to metamorphosis, length at metamorphosis and weight at metamorphosis for the different diet treatments.

RESULTS

Feeding treatment led to a significant difference in the amount of time it took for tadpoles to reach metamorphosis (H(2) = 22.68, $p < 0.05$) (tab. 4). Specifically, post-hoc pairwise comparisons revealed that tadpoles fed mustard greens had significantly longer larval stages (more days before metamorphosis) than those fed on the other two diets ($p < 0.05$) (tab. 5), whereas there was no difference in time to metamorphosis between the tadpoles fed spirulina or those fed shrimp (fig. 2). Larval diet also affected length and weight at metamorphosis, (H(2) = 13.50, $p < 0.05$) and (H(2) = 9.45, $p < 0.05$) respectively (tab. 4). Similarly to days to metamorphosis, pairwise post-hoc analyses showed that the mustard green diet led to the significant differences in length and weight of metamorphs ($p < 0.05$) (tab. 5), while no significant difference in either length or weight was found between the groups fed shrimp and spirulina (fig. 2). Specifically, metamorphs fed mustard greens were shorter in length and weighed less than those fed the other diets. Average and standard deviations among treatments are shown in tab. 6. Four tadpoles died before metamorphosis, therefore we ran a Fisher exact test to analyse if survivorship to metamorphosis differed among treatments. No statistically significant differences in survivorship among diet treatments were found ($p = 0.53$) (tab. 2).

Table 4. Results of Kruskal-Wallis tests.

	H	degrees of freedom	*p* value
Days to metamorphosis	22.6774	2	0.00012
Length at metamorphosis	13.5045	2	0.00117
Weight at metamorphosis	9.4500	2	0.00887

DISCUSSION

Diet treatments of spirulina and atyid shrimp both resulted in similar shorter times to and larger size at metamorphosis, suggesting these locally available foods are better than mustard greens for raising larvae of *Mantidactylus betsileanus*. This does not mean, however, that they are necessarily the best diets. It is possible that other food options, such as manufactured tropical fish flakes and commercial tadpole diets, could result in improved individual fitness or that using a variety of foods would result in better growth and development. For instance, Altig *et al*. (2007) note that while generalized or omnivorous feeders may only consume low levels of animal material as part of their diet, it is this highly nutritious component that contributes to high production and rapid development, so while feeding only mustard greens slowed development and produced smaller frogs in our study, combining greens with a portion of atytid shrimp or other animal material could produce very different results.

Table 5. A. Post-hoc pairwise comparisons of days to metamorphosis among 3 diets. **B.** Post-hoc pairwise comparisons of length at metamorphosis among 3 diets. **C.** Post-hoc pairwise comparisons of weight at metamorphosis among 3 diets.

A			
		Greens	Shrimp
	Shrimp	0.0001	
	Spirulina	0.0011	0.3216
B			
		Greens	Shrimp
	Shrimp	0.0044	
	Spirulina	0.0214	1.0000
C			
		Greens	Shrimp
	Shrimp	0.0260	
	Spirulina	0.0450	1.0000

It should also be noted that the traits that we measured do not encompass the entire range of characteristics that determine the fitness of an individual tadpole. Weight and length at start of metamorphosis or throughout the larval stage were not recorded, and these traits could also be dependent on diet and have an effect on fitness of tadpoles and metamorphs. Another consideration is that the growth of tadpoles is highly dependent on temperature, and while the varying temperature in our study was the same between treatments since they were run at the same time and in the same location, it is possible that under different environmental conditions the three diet treatments could produce different results.

Dietary protein has been shown to have a significant effect on the development of amphibian larvae. Though the optimal captive diet and its protein content likely reflect the natural history of the species being maintained, larval diets have been tested on a number of anuran species and those with high protein contents have resulted in better growth and development (Steinwascher & Travis, 1983; Carmona-Osalde *et al*., 1996; Martins *et al*., 2013). It is not so surprising then that spirulina and atyid shrimp, both of which have protein contents upwards of 50% (Ciferri, 1983; Mugo-Bundi *et al*., 2015), resulted in shorter times to and larger size at metamorphosis than mustard greens, which have a protein content of less than 3% (USDA, 2014). Although mustard greens in our study did not affect survivorship of *Mantidactylus bestileanus* and various other leafy greens have been used and recommended as a captive diet for tadpoles in literature (Briggs & Davidson, 1942; Martin, 1991; Banks *et al.*, 2008; Tyler, 2009), there are clearly better options available for feeding tadpoles. It should be noted, however, that we did not measure the nutrient content of our experimental diets, and so cannot directly infer that the higher protein diets led to better growth and development in our study.

Mantidactylus betsileanus has been assessed by the IUCN Red List as Least Concern and seems to be widespread but it is also clear that the species is composed of a complex of undescribed species, many of which are thus far known from only one or two localities (Glaw & Vences, 2007; Vences & Nussbaum, 2008). While the status in the wild and larval life histories of these candidate *Mantidactylus* species is unknown, the results from our study suggest that if *ex situ* conservation breeding programmes are needed in the future, a starting point for a captive larval diet could be based on spirulina and atyid shrimp. However, future studies that measure the nutritional composition of different diets and how the nutrients in those diets are assimilated by tadpoles would expand our understanding of optimal diets for captive breeding. With this in mind, we hope to see future captive husbandry studies conducted on other Malagasy anuran species by the international *ex situ* community which could help inform conservation breeding programmes for other poorly known and potentially threatened species.

Table 6. Average and standard deviation for each treatment.

Treatment	Days to metamorphosis		Weight at metamorphosis		Length at metamorphosis	
	AVG	STDEV	AVG	STDEV	AVG	STDEV
Spirulina	108.00	6.83	0.17	0.04	11.93	0.45
Atyid shrimp	103.25	17.18	0.17	0.04	12.42	1.83
Greens	171.31	38.25	0.12	0.04	10.54	1.13

RESUMÉ

Des programmes d'élevage en captivité des amphibiens sont de plus en plus nécessaires en vue du déclin et des risques d'extinction, auquel cette classe animale est confrontée à court terme. De plus, les principaux aspects de l'élevage, tels que l'alimentation des larves, continuent d'être négligés, parfois même totalement inconnu. A Madagascar, il peut être difficile d'adopter ces programmes, en raison de l'indisponibilité de régimes spécifiques aux têtards. Nous avons testé trois régimes, disponibles localement à Madagascar : la moutarde brune, l'algue spiruline, ainsi que des crevettes de la famille des Atyidae, sur les larves de *Mantidactylus betsileanus*, et enregistré leur croissance et leur développement. Il en résulte, que les têtards nourris aux feuilles de moutarde ont eu un développement plus long et leur métamorphose s'est effectuée à une taille plus petite. Cependant, aucune différence du taux mortalité des têtards n'a été observée entre les différents programmes. Ces résultat suggèrent que les moutarde brune sont une source de nourriture pauvre, tels que la spiruline et les crevettes de la famille des Atyidae, sont de meilleurs choix que les légumes ou végétaux pour l'élevage de *M. betsileanus* et des espèces similaires. Bien que d'autres alternatives comme les aliments industriels destinés spécialement aux têtards ou poissons pourraient donner des résultats de meilleures qualités.

ACKNOWLEDGEMENTS

We wish to express our gratitude to Woodland Park Zoo, Biopat, Tree Walkers International, Understory Enterprises, Cleveland Metroparks Zoo Africa Seed Grants program, and the 2013 Sustainable Amphibian Conservation of the Americas Symposium for their financial support of husbandry research at Mitsinjo leading up to and during the duration of this study. We also would like to recognize Jennifer Pramuk and Brian Gratwicke for their help in developing and improving the methodology of the experiment. Renaud Boulenger kindly improved our French abstract. This project was carried out through a contract of collaboration between the Malagasy authority of the Direction Générale des Forêts, Association Mitsinjo, and the IUCN SSC Amphibian Specialist Group of Madagascar.

LITERATURE CITED

Altig, R., Whiles, M.R., Taylor, C.L. (2007). What do tadpoles really eat? Assessing the trophic status of an understudied and imperilled group of consumers in freshwater habitats. *Freshwater Biology*, 52: 386-395.

Altwegg, R., Reyer, H. (2003). Patterns of natural selection on size at metamorphosis in water frogs. *Evolution*, 57(4): 872-882.

Álvarez, D., Nicieza, G. (2002). Effects of temperature and food quality on anuran larval growth and metamorphosis. *Ecology*, 16: 640-648.

Andreone, F., Randriamahazo, H. (2008). *Sahonagasy Action Plan. Conservation Programs for the Amphibians of Madagascar / Programmes de conservation pour les amphibiens de Madagascar.* Museo Regionale di Scienze Naturali, Conservation International, IUCN/SSC Amphibian Specialist Group. Turin, Italy.

Arnoult, R., Razarihelisoa, M. (1967). Contribution a l'étude des batraciens de Madagascar: Le genre *Mantidactylus* adultes et forms larvaires de *M. betsileanus* (Blgr.), *M. curtus* (Blgr.) et *M. alutus* (Peracca). *Bulletin du Muséum national d'Histoire Naturelle* (Paris), 39: 471-487.

Banks, C., Lau, M., Dudgeon, D. (2008). Captive management and breeding of Romer's tree frog *Chirixalus romeri*. *International Zoo Yearbook*, 42: 99-108.

Bletz, M.C., Rosa, G., Andreone, F., Courtois, E.A., Schmeller, D.S., Rabibisoa, N.H.C., Rabemananjara, F.C.E., Raharivololoniaina, L., Vences, M., Weldon, C., Edmonds, D., Raxworthy, C.J., Harris, R.N., Fisher, M.C., Crottini, A. (2015). Widespread presence of the pathogenic fungus *Batrachochytrium dendrobatidis* in wild amphibian communities in Madagascar. *Scientific Reports*, 5. 8633.

Blommers-Schlösser, R.M.A. (1979). Biosystematics of the Malagasy frogs, I. Mantellinae (Ranidae). *Beaufortia*, 29: 1-77.

Briggs, R., Davidson, M. (1942). Some effects of spinach-feeding on *Rana pipiens* tadpoles. *Journal of Experimental Zoology*, 90: 401-411.

Carmona-Osalde, C., Olvera-Novoa, M.A., Rodríguez-Serna, M., Flores-Nava, A. (1996). Estimation of the protein requirement for bullfrog (*Rana catesbeiana*) tadpoles, and its effect on metamorphosis ratio. *Aquaculture*, 141: 223–231.

Christie, M.R., Marinea, M.L., French, R.A., Blouin, M. S. (2012). Genetic adaptation to captivity can occur in a single generation. *Proceedings of the National Academy of Sciences*. 109: 238-242.

Ciferri, O. (1983). Spirulina, the edible microorganism. *Microbiological Reviews*, 1983: 551-578.

Edmonds, D., Rakotoarisoa, J.C., Dolch, R., Pramuk, J., Gagliardo, R., Andreone, F., Rabibisoa, N., Rabemananjara, F., Rabesihanaka, S., Robsomanitrandrasana, E. (2012). Building capacity to implement conservation breeding programs for frogs in Madagascar: Results from year one of Mitsinjo's amphibian husbandry research and captive breeding facility. *Amphibian and Reptile Conservation*, 5: 57-69.

Formanowicz Jr., D., Brodie Jr., E. (1982). Relative palatabilities of members of a larval amphibian community. *Copeia*, 1982: 91-97.

Gagliardo, R., Crump, P., Griffith, E., Mendelson, J., Ross, H., Zippel, K. (2008). The principles of rapid response for amphibian conservation, using the programmes in Panama as an example. *International Zoo Yearbook*, 42: 125-135.

García, G., Bock, L., Earle, S., Berridge, R., Copsey, J. (2008). Captive breeding as a tool for the conservation of Malagasy amphibians: how ready are we to respond to the need? *In*: Andreone, F. (ed.) *A Conservation Strategy for the Amphibians of Madagascar.* Monografie XLV. Museo Regionale di Scienze Naturali, Torino, Italy: 321-342.

Glaw, F., Vences, M. (2007). *A field guide to the amphibians and reptiles of Madagascar.* Third edition. Vences & Glaw Verlag, Cologne.

Griffiths, R., Pavajeau, L. (2008). Captive breeding, reintroduction, and the conservation of amphibians. *Conservation Biology*, 22: 852-861.

Gosner, K. (1960). A simplified table for staging Anuran embryos and larvae with notes on identification. *Herpetologica*, 16: 183-190.

Leips, J., Travis, J. (1994). Metamorphic responses to changing food levels in two species of Hylid frogs. *Ecology*, 75: 1345-1356.

Martin, D. (1991). Captive husbandry as a technique to conserve a species of special concern, the Yosemite toad. *Proceedings of the Northern California Herpetological Society:* Conference on captive propagation and husbandry of reptiles and amphibians.Northern California Herpetological Society, Sacramento, CA, 6: 16-32.

Martins, F.M.S., Oom, M. d. M., Rebelo, R., Rosa, G. M. (2013). Differential effects of dietary protein on early life-history and morphological traits in natterjack toad (*Epidalea calamita*) tadpoles reared in captivity. *Zoo Biology,* 32: 457-462.

McCallum, M.L., Trauth, S.E. (2002). Performance of wood frog (*Rana sylvatica*) tadpoles on three soybean meal – corn meal rations. *Pod@rcis*, 3: 78-85.

McWilliams, D. (2008). Nutrition recommendations for some captive amphibian species (Anura and Caudata). Canadian Association of Zoos and Aquariums Nutrition Advisory and Researh Group, Guelph. Available from http://www.caza-narg.ca/ref/amphibian%20nutrition%20report%20CAZA%202008.pdf (accessed August 2015).

Michaels, C., Gini, B., Preziosi, R. (2014). The importance of natural history and species-specific approaches in amphibian *ex-situ* conservation. *The Herpetological Journal*, 24: 135-145.

Mugo-Bundi, J., Oyoo-Okoth, E., Ngugi, C., Manguya-Lusega, D., Rasowo, J.,Chepkirui-Boit, V., Opiyo, M., Njiru, J. (2015). Utilization of *Cardinanilotica* (Roux) meal as a protein ingredient in feeds for Nile tilapia (*Oreochromis niloticus*). *Aquaculture Research*, 46: 346-357.

Newman, R. (1998). Ecological constraints on amphibian metamorphosis: interactions of temperature and larval density with responses to changing food level. *Oecologia*, 115: 9-16.

Perl, R. G.B., Nagy, Z.T., Sonet, G., Glaw, F., Wollenberg, K.C., Vences, M. (2014). DNA barcoding Madagascar's amphibian fauna. *Amphibia-Reptilia*, 35: 197-206.

Pessier, A. (2010). Amphibian nutrition. In: *Amphibian Biology, Conservation, and Management.* AZA Professional Training Program. Association of Zoos and Aquariums, Silver Spring, MD: 1-16.

Pramuk, J., Gagliardo, R. (2012). General amphibian husbandry. *In*: Poole, V.A., S. Grow (eds.). *Amphibian Husbandry Resource Guide.* Edition 2.0. Association of Zoos and Aquariums, Silver Spring, MD: 4-59.

Scheele, B., Hunter, D., Grogan, L., Berger, L., Kolby, J., McFadden, M., Marantelli, G., Skerrat, L., Driscoll, D. (2014). Interventions for reducing extinction risk in chytridiomycosis-threatened amphibians. *Conservation Biology*, 28: 1195-1205.

Scheld, S., Bina Perl, R. G., Rauhaus, A., Karbe, D., Van Der Straeten, K., Hauswaldt, J.S., Randrianiaina, R.D., Gawor, A., Vences, M., Ziegler, T. (2013). Larval morphology and development of the Malagasy frog *Mantidactylus betsileanus*. *Salamandra*, 49: 186-200.

Steinwascher, K., Travis, J. (1983). Influence of food quality and quantity on early larval growth of two anurans. *Copeia*, 1983: 238-242.

Tapley, B., Bradfield, K. S., Michaels, C., Bungard, M. (in press). Amphibians and conservation breeding programmes: do all threatened amphibians belong on the ark? *Biodiversity Conservation*, DOI: 10.1007/s10531-015-0966-9.

Tyler, M. (2009). *Frogs and toads as experimental animals.* ANZCCART. The University of Adelaide, South Australia.

United States Department of Agriculture (USDA). (2014) *National Nutrient Database for Standard Reference Release 27. Full Report (All Nutrients): 11271, Mustard greens.* The National Agricultural Library. Agricultural Research Service (accessed February 2015).

Vences, M., Nussbaum, R. (2008). *Mantidactylus betsileanus. In:* IUCN 2014. *IUCN Red List of Threatened Species.* Version 2014.2. Available from www.iucnredlist.org (accessed March 2015).

Vieites, D.R., Wollenberg, K.C., Andreone, F., Köhler, J., Glaw, F., Vences, M. (2009). Vast underestimation of Madagascar's biodiversity evidenced by an integrative amphibian inventory. *Proceedings of the National Academy of Sciences,* 106: 8267-8272.

Wells, K.D. (2007). *The ecology and behavior of amphibians.* University of Chicago Press, Chicago.

Werner, E. (1986). Amphibian metamorphosis: Growth rate, predation risk, and the optimal size at transformation. *The American Naturalist*, 128: 319-341.