



Relating natural climate and phenology to captive husbandry in two midwife toads (*Alytes obstetricans* and *A. cisternasii*) from different climatic zones

Christopher J. Michaels^{1*}, Michael Fahrbach², Luke Harding¹, Zoe Bryant¹, Joseph-Smilely Capon-Doyle¹, Sebastian Grant¹, Iri Gill¹, Benjamin Tapley¹

¹ Zoological Society of London, Regent's Park, London NW1 4RY, UK

² Niedernhallerstraße 10, 74653 Criesbach, Germany

Captive husbandry and breeding may be pivotal to the successful conservation of many amphibian species, with captive stock providing research subjects, educational tools and animals for release into the wild. Husbandry protocols are missing for many species and sub-optimal for many more, which may limit the success of captive breeding attempts. It has been suggested that observations and environmental data taken from species in nature may be used to infer optimal captive conditions for amphibians. For species where data from the wild are not available, 'analogue', that is closely related but more accessible species, may be used as surrogates to inform captive husbandry to some degree. These hypotheses, although logically cogent, are not well tested in amphibians. In particular, the suitability of analogue species based on some knowledge of basic ecology and biology is frequently not assessed. We show that captive husbandry requirements and breeding stimuli correlate with field data and phenology in wild populations of the midwife toads *Alytes obstetricans* and *A. cisternasii*. In particular, the provision of hot summer temperatures following a cold brumation period of suitable duration may be important for breeding the western-central European *A. obstetricans*. Conversely, the Iberian *A. cisternasii* responds to hot summer temperatures with a rest period and reproduces in the cooler autumn and winter months. Brooding success was highly variable in *A. obstetricans* and smaller than records from wild toads, possibly due to the young age of breeding stock. Clutch size was similar in *A. cisternasii* to records from wild counterparts. Although specific breeding triggers and annual temperature requirements are likely to vary between localities for both species, these observations provide some useful data on the indoor breeding of both species. Our results also highlight the relevance of field data in designing captive husbandry protocols, while illustrating the inappropriateness of using one species as an analogue for the other in terms of husbandry requirements unless basic aspects of natural history, ecology and phenology can be shown to be broadly similar.

INTRODUCTION

Captive breeding, for release, research and for educational purposes, is an important facet of amphibian conservation (Gascon *et al.*, 2007). With more than two-thirds of amphibians threatened with extinction (IUCN, 2015) and more than 500 species thought to require urgent *ex situ* intervention (Zippel *et al.*, 2006), it is important that husbandry practices are optimised. Unfortunately, many amphibian species have not been maintained in captivity before and initial attempts may be partly or wholly unsuccessful (Gagliardo *et al.*, 2008; Michaels *et al.*, 2014; Tapley *et al.*, 2015). Amphibians, like all organisms, exhibit adaptations in physiology, ecology, behaviour and phenology to their natural habitat. Moreover, amphibians are typically highly sensitive to their immediate environment and as such have highly specific requirements for survival and reproduction, both in the field and in

captivity (Michaels *et al.*, 2014; Tapley *et al.*, 2015). Therefore it has been suggested that, from observations of the habitat, inferences can be made about the captive husbandry needs of amphibians (Tapley & Acosta, 2010; Michaels *et al.*, 2014). This concept has been further extended to suggest that, in the absence of field data on a focal species, observations of a closely related, or ‘analogue’, species may be used as a proxy (Michaels *et al.*, 2014). Caveats concerning the selection of analogue species are frequently overlooked, however, and the importance of understanding similarities and differences between focal and candidate analogue species may be forgotten. Despite frequent practical application (pers. obs.), there have been few investigations that explore the linked concepts of field data informed captive husbandry and analogue species (see Michaels *et al.* (2014) for discussion) in practice.

We present observations from two species of midwife toads, *Alytes obstetricans* (Laurenti, 1768) and *A. cisternasii* Boscá, 1879, relating wild micro- and macro-climate observations to the development of successful captive husbandry practice in these two closely related, but ecologically disparate, species. We also use them to illustrate the dangers of using poorly considered analogue species.

The genus *Alytes* Wagler, 1830 comprises five recognised species distributed across western and the western part of central Europe and north Africa. Midwife toads are so-called because male toads brood egg clutches, which they carry on their hind legs (fig. 1.A, B), until they are ready to hatch into tadpoles. The common midwife toad, *A. obstetricans*, is a terrestrial species widely distributed across western Europe, where it inhabits temperate climatic zones with warm summers and cold winters (Bosch *et al.*, 2009). The Iberian midwife toad, *A. cisternasii*, by contrast, is more fossorial and is found in Mediterranean climates in southwest Europe where it experiences very hot, dry summers and mild, wet winters (Beja *et al.*, 2009). These different climates have shaped divergent phenologies in these species in nature (Marquez, 1992). Both species are increasingly threatened in the wild with decreasing populations (Beja *et al.*, 2009; Bosch *et al.*, 2009) and are held at ZSL London Zoo as part of ongoing collaborative research projects investigating captive husbandry and biology.

MATERIALS AND METHODS

Collection of field data

The temperature of two refugia used by *Alytes obstetricans* during the reproductive season was measured in throughout May, June and July in garden habitat in Baden Württemberg, south-western Germany. The presence of toads was noted and temperatures were taken immediately adjacent to animals using a Cason CA380 infrared thermometer or, in the absence of toads on the positions where they had previously been discovered. Refugia were lifted as little as possible and temperatures measured immediately to avoid changes in temperature caused by disturbance. As infra-red thermometers measure surface temperature rather than air temperature there is sufficient lag time between disturbing refugia and significant temperature change to collect accurate readings. Due largely to the fossorial and therefore inaccessible behaviour of *A. cisternasii* as well as its more limited distribution, field data from the micro-habitat of this species were not available. However, general climatic data for the Algarve region (World Weather Online, 2015) were used alongside observations of the natural history of the species to inform captive husbandry parameters.

Captive husbandry of toads

Captive colonies of *Alytes obstetricans* from three French Pyrénéan localities (Lac de Lhurs 42°55'19.4"N, 0°42'15.2"W; (14 males, 8 females; 14.8), Lac d'Arlet 42°50'21.1"N, 0°36'53.4"W (10.10) and Lac d'Ansabere 42°53'15.2"N, 0°42'30.6"W (10.8)) and of *A. cisternasii* (2.2) of stock originating in the Algarve, Portugal, were maintained at ZSL London Zoo. *Alytes obstetricans* were collected as tadpoles in 2012; *Alytes cisternasii* were captive bred in the winter of 2011/12. Toads were sexed by the larger size of females (Bosch and Marquez, 1996; Marquez *et al.*, 1997) and the presence of ova visible through the abdominal wall (fig. 1.C). All enclosures were held within climate-controlled facilities. The SVL (snout-vent length) of toads was measured using calipers.

Alytes obstetricans was maintained in several different enclosure types throughout the year according to husbandry requirements. Throughout the autumn and spring, up to twelve toads were maintained in 45x45x45 cm vivaria. Toads were kept on a substrate of gravel standing in a shallow layer of water, with the gravel held back at one side by a raised glass strip to provide a pond. Enclosures were sprayed with a misting system every three days and weekly water changes were used to flush detritus out of the gravel and to maintain good water quality. *Tradescantia fluitans* was grown hydroponically in the gravel to provide shelter and to extract nitrogenous waste. Stacks of broken unglazed tiles and brick were piled on top of the substrate to produce numerous crevices with

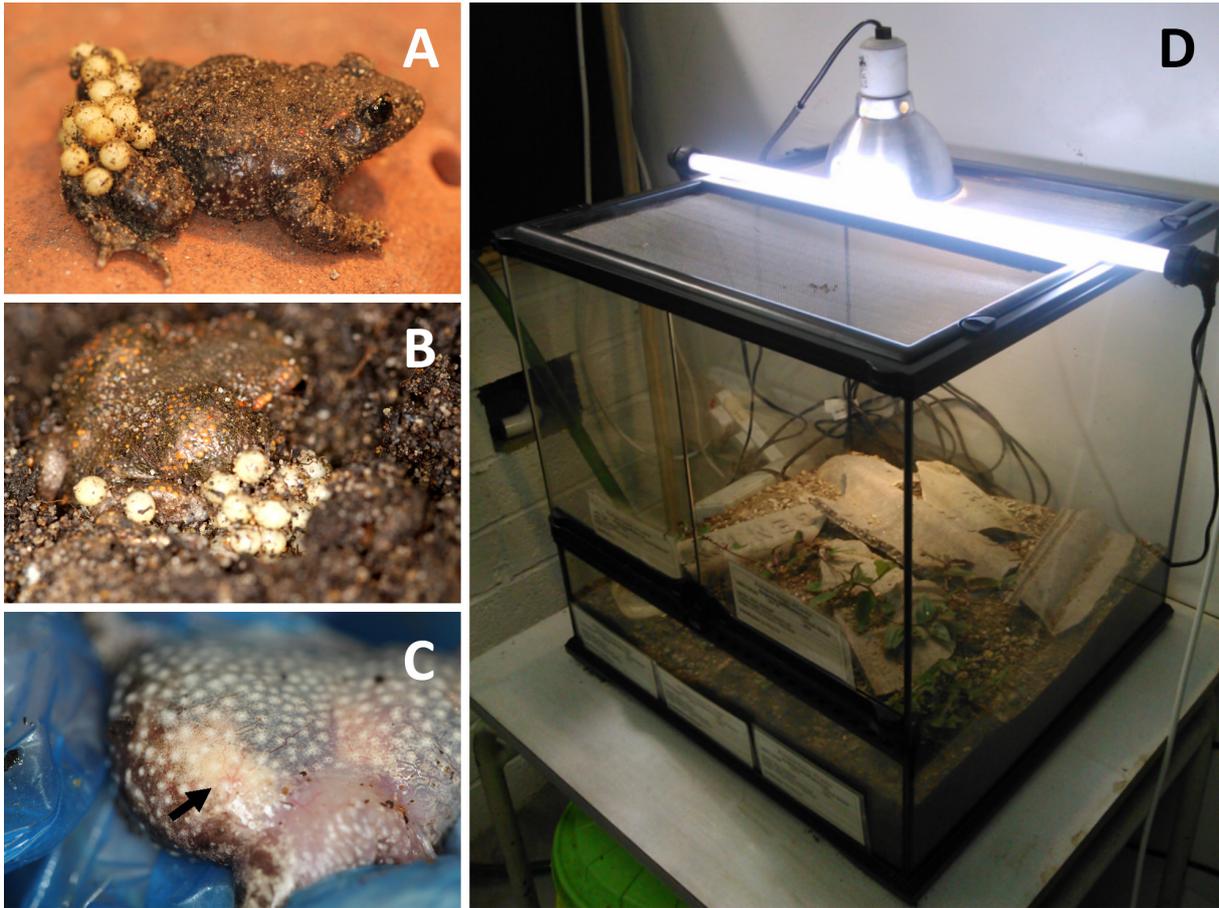


Figure 1. A. Male *Alytes obstetricans* carrying egg clutch; B. *A. cisternasii* male carrying egg clutch; C. female *A. obstetricans* with eggs (indicated by black arrow) visible in abdomen; D. Enclosure style used to house both *A. cisternasii* and *A. obstetricans* during the summer, with incandescent and T5 fluorescent lamps providing heat and UVB, UVA, and visible light.

varying dampness created by capillary action. Temperatures during these periods are summarised in fig. 2.

During the winter, toads were moved to small plastic boxes (38x25x15 cm) with a substrate composed of 10 parts sterile loamy topsoil, 2 parts coco fibre and 1 part sharp sand, dampened with water. Stacks of broken unglazed tile were provided as shelter and a small shallow water dish was present. *A. obstetricans* is found in regions where winter temperatures fall well below freezing, but the toads are not frost resistant and use hibernacula where they are protected from freezing (Marquez, 1992). We therefore held these boxes in a refrigerated unit between 0.5 and 3°C at which temperatures they became entirely torpid, moving only when disturbed and then only slowly. Toads from Lac d'Arlet and Lac de Lhurs were brumated between 29 December 2014 and 03 March 2015 (fig. 2) and those from Lac d'Ansabere between 6 January 2014 and 30 March 2015. After the brumation period, temperatures were increased over 24 hours to 10-12°C (fig. 2) and toads returned to autumn/spring enclosures, as described above. On the 20 March 2015, temperatures were increased to 16°C (fig. 2).

Finally, on the 30 April 2015, toads were moved to summer enclosures (fig. 1.D). These enclosures consisted of 45x45x45cm Exo Terra vivaria (Exo Terra, Germany) with a sandy substrate composed of 4:1:2 Breedon 'golden amber gravel' (Breedon specialist aggregates, Derby, UK): sharp sand: peat-free compost. Stacks of broken unglazed tiles were again provided for shelter. Half the males and half the females of each population were housed as a group with a spot light (60 W incandescent reflector lamp), providing a thermal gradient from 31°C on the exposed surface of upper tiles, to 18-21°C in substrate under tiles and crevices. Temperatures in between these two extremes were available. This was designed to mimic the sunny rock piles, banks and drystone walls that this species frequently uses during the summer in the wild and to replicate the temperatures measured in nature (see data presented in tab. 1). Toads maintained without access to these lamps were held at the same ambient temperature of 18-21°C. At all times of the year, other than winter brumation, UVB emitting lamps (Arcadia D3+ 12% UVB lamps) were used to provide a UV index (UV_i, a unitless measure of biologically relevant UVB exposure; see Michaels & Preziosi (2014) for a full discussion of this measure) gradient of 0-1 across each enclosure. UVB radiation has been shown to have some benefits in the husbandry of closely allied bombinatorid

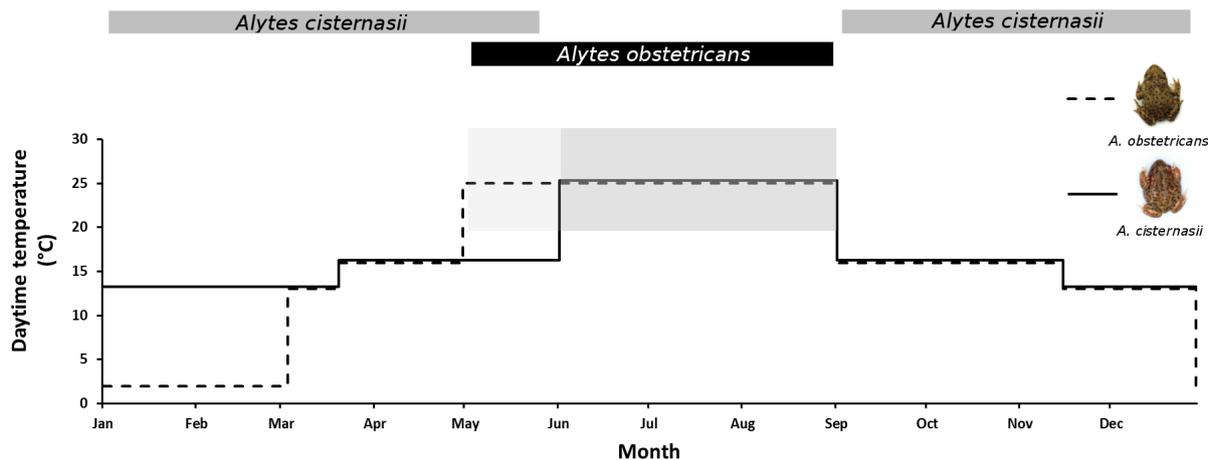


Figure 2. Median diurnal temperature regimens used for Lac de Lhurs and Lac d'Arlet populations of *A. obstetricans* and *A. cisternasii* at ZSL London Zoo. The grey shaded area across the lines between May and September represents the limits of the thermal gradient provided during these months; the darker section represents both species, the lighter section only *A. obstetricans*. All enclosures experienced a 5°C temperature drop at night apart from during the winter (lowest marked temperatures). The spawning periods for *A. obstetricans* (black) and for *A. cisternasii* (grey) are indicated by the bars above the graph. November-December are data for 2014, all other months for 2015. Lac d'Ansabere *A. obstetricans* began brumation one month after the other populations, but was otherwise identical.

anurans (Michaels *et al.*, 2015), as well as in other anuran amphibians (Verschooren *et al.*, 2011; Tapley *et al.*, 2015a). UVi gradients were designed to correlate with temperature gradients as closely as possible.

Therefore, all *A. obstetricans* toads were subject to identical Autumn and Winter conditions and then brumated at the same temperature (Lac D'Arlet/Lac De Lhurs = 65 days, Lac D'Ansabere = 57 days). All *A. obstetricans* were exposed to the same ambient temperature during the summer period in the same types of enclosure, but half of each locality population was provided with a basking spot and the other half was not.

A. cisternasii is a fossorial species native to a more Mediterranean climate with milder winters. In winter, this species was therefore exposed to minimum temperatures of 10-12°C and in summer moved to enclosures identical to those used for *A. obstetricans* at this time of year, except with deeper substrate (10 cm). The temperature regimen used for this species is shown in fig. 2.

Water dishes were provided in summer enclosures for soaking and tadpole deposition. When tadpoles were deposited by brooding males, they were transferred to 50x30x30 cm aquaria held at 18-19°C and filled with 9:1 RO: tap water (Alkalinity 130 mg/L; pH 7.2) filtered with airstream sponge filters (various brands and models). *Egeria densa* stems were provided to help maintain water quality. Tadpoles were fed ad libitum on a diet composed of homogenised Spirulina, freeze-dried shrimp, freeze-dried Tubifex, fish-flake and grass pellet, and received at least one 10% water change per week.

All toads were fed twice weekly on small insects including crickets (*Gryllus bimaculatus* and *Acheta domestica*), cockroaches (*Blaptica dubia*), bean weevils (*Callosobruchus maculatus*), and locusts (*Schistocerca gergaria*) of appropriate sizes. All prey items (with the exception of *C. maculatus*, which do not feed as imagos) were gut-loaded for at least 48 hours on fresh fruit and vegetables, and Spirulina flake. Prey items were dusted with Nutrobal (VetArk) vitamin and mineral supplement immediately before being offered to toads. Powder-free nitrile gloves were used to handle toads or any contaminated objects. All solid waste from toads was incinerated and all liquid waste filtered to remove organic debris, then disinfected following the manufacturer's guidelines with Anigene HLD4V (Medimark Scientific, Sevenoaks, Kent, UK) before being discharged into municipal sewers, in order to minimise the risk of accidental pathogen release.

RESULTS

Field measurements of *A. obstetricans* refugium microclimate temperatures are presented in tab. 1. Toads were no longer present in focal refugia once temperatures climbed consistently above 40°C (July readings; tab. 2). At this point, the substrate beneath refugia had also become increasingly dry. Toads had remained in the same refugia following numerous brief disturbances at lower temperatures and so it is unlikely that a threshold of disturbance was crossed entirely coincidentally with rising temperatures. In either case, toads were not found exposed to

Table 1. Temperature measured in two refugium microclimates of *Alytes obstetricans* in Baden Württemberg, Germany throughout the breeding season.

Date (morning / afternoon)	Temperature (°C)		Toads present?
	Refugium 1 (stone slabs and piles covered with sempervivum on sand)	Refugium 2 (Rocks on pond liner on soil)	
03/05/2015 (afternoon)	23.5	32.2	+
24/05/2015 (morning)	25.2	34.5	+
05/06/2015 (morning)	27.6	39.7	+
08/06/2015 (afternoon)	24.1	33.1	+
20/06/2015 (afternoon)	19.8	27.7	+
02/07/2015 (afternoon)	40.6	49.1	-
03/07/2015 (afternoon)	39.5	45.8	-
04/07/2015 (morning)	23.9	26.5	-
05/07/2015 (afternoon)	43.2	52.1	-

temperatures at this level. Macro-climate data for the range of *A. cisternasii* shows a typical Mediterranean climate with hot, dry summers and mild, wet winters.

In captivity, *A. cisternasii* reproduced following a drop in temperature at the end of summer and continued to reproduce throughout the autumn, winter and spring (fig. 2). *A. obstetricans* reproduced after an increase in temperature to values within the range measured in the field, and reproduced throughout the early summer period (fig. 2). Groups of each *A. obstetricans* population of the same number and sex ratio maintained without the thermal gradient generated by the incandescent lamp did not breed (tab. 2). Additionally, toads from Lac d'Ansabere, which were brumated for a shorter period, did not reproduce in any enclosure type. In both species, calling and first reproduction occurred within 24 hours of the temperature change. *A. obstetricans* exhibited a higher rate of clutch abandonment, with five of eight clutches (treating one double clutch as a single clutch for this purpose) abandoned before development was complete (for *A. cisternasii*, one out of four was abandoned) although more individuals of this species meant that more clutches were also produced overall (see tab. 2). In at least one case, this was associated with substrate temporarily becoming too dry.

The incubation period for successful *A. obstetricans* clutches was 12-18 days and 33-95 days for *A. cisternasii*. In the latter species, clutches were carried at different temperatures, with those brooded during the coolest months having a much longer incubation period (see tab. 2). *A. obstetricans* deposited 21-24 tadpoles which measured 13-15 mm total length upon hatching. Clutch viability in *A. obstetricans* (i.e. number of live tadpoles hatching from a clutch) was 71-78%, excluding a probable double clutch with an overall hatch rate of 45% likely due to infertility of one clutch. *A. cisternasii* deposited 17-27 viable tadpoles, with a clutch viability of 40%. Tadpoles of *A. cisternasii* measured approximately 8 mm total length upon hatching.

DISCUSSION

Alytes species can be roughly split into two ecological groups, those whose reproductive period in nature is defined mainly by hydroperiod and those with a reproductive period defined mainly by temperature, although both variables have some influence to a greater or lesser degree (Marquez, 1992). Based on observations of reproduction in the field, *A. obstetricans* belongs to the latter and *A. cisternasii* to the former (Marquez, 1992). *A. cisternasii* is adapted to arid summers and wet, mild winters (although it may stop reproducing during very cold weather when it is too cold for activity) and so is stimulated to breed by a temperature drop and correlated increase in moisture levels, both in nature (Marquez & Jiménez, 1984; Jiménez, 1988; Marquez & Verrell, 1990; Marquez, 1992; Masó & Pijoan, 2011) and in captivity (this study). Conversely, wild Iberian midwife toads cease breeding in the summer, when water availability drops (Marquez & Verrell, 1990; Marquez, 1992; Masó & Pijoan, 2011); similarly in captivity, an increase in temperature and drying of the substrate produced a non-reproductive phase in

Table 2. Breeding trial information for *Alytes obstetricans*. Zoological Information Management System (ZIMS) identification number; SVL (snout-vent length) provided as mean \pm standard deviation; Sex ratio (as the “number of males . number of females”) giving per split breeding group; Yes, Y; No, N.

Species	Geographic origin	ZIMS	SVL (cm)	Sex ratio	Brumation period (days)	Hot summer period	Spawning	Clutch date	Carried to term	Clutch size (<i>n</i> hatched)	Brooding period (days)
<i>Alytes obstetricans</i>	Lac de Lhurs	G00063	4.02 (0.24)	7.4	65	Y	Y	11/05/2015	N	21 (0)	<3
								16/06/2015	N	20 (0)	
								19/06/2015	N	26 (0)	<3
<i>Alytes obstetricans</i>	Lac d'Arlet	G00065	4.36 (0.15)	5.5	65	Y	Y	02/05/2015	N	8 (N/A)	4
								15/05/2015	Y	32 (24)	12
								15/05/2015	Y	27 (21)	13
								15/05/2015	N	Unknown	<2
								12/06/2015	Y	61 (28)* ¹	18
<i>Alytes obstetricans</i>	Lac d'Ansabere	G00064	4.15 (0.18)	5.3	57	Y	N				
									N		
<i>Alytes cisternasii</i>	Algarve	G01439 - G01442	3.44 (0.34)	2.2	N/A	Y	Y	04/11/2014	N	Unknown	Unknown
								10/02/2015	Y	54 (14)	95* ²
								30/04/2015	Y	65 (27)	33
								01/06/2015	Y	49 (29)	34

*¹ probable double clutches; *² clutch brooded over winter at coolest temperatures

this species. The clutch sizes reported here are within the ranges already described for *A. cisternasii* (Crespo, 1979; Malkmus, 1983; Marquez, 1993; 1996; Wells *et al.*, 2015). However, wild clutch sizes for *A. obstetricans* may be larger than those reported here, although other authors report more similar clutch sizes (Crespo, 1979; Bucholz, 1989; Galan *et al.*, 1990; Marquez, 1993; 1996; Reading & Clarke, 1988; Wells *et al.*, 2015 and references therein). In *A. muletensis*, captive and wild clutch sizes are also broadly similar (Wells *et al.*, 2015 and references therein). Age and body size may be important determinants of anuran clutch size, including in *Alytes* (Marquez, 1992; Marquez, 1993; Böll & Linsenmair, 1998). The *A. obstetricans* used in the present study metamorphosed in 2012 and hence were only 2 years post metamorphosis at the time of reproduction. These animals were also smaller than that reported for wild breeding populations of toads (see tab. 2; Marquez, 1993; Bosch & Marquez, 1996; Marquez *et al.*, 1997). These two factors may have caused the smaller clutch sizes produced in this species. Conversely, our *A. cisternasii* were similar in size (tab. 2) to wild populations (Marquez, 1993; Bosch & Marquez, 1996; Marquez *et al.*, 1997), although our sample is smaller for this species.

In *A. obstetricans*, parental success (i.e. proportion of clutches hatching into tadpoles; 71-78% in this study, tab. 2) was similar to that reported for wild animals (mean 74%, Marquez, 1993), but much lower for *A. cisternasii* (40% in this study, tab. 2), which in nature is reported to have a mean success rate of 89% (Marquez, 1993). It is unclear why hatching success was lower in *A. cisternasii*, but unhatched eggs were almost all infertile, showing no development at all, so the issue is more likely to be to do with male fertility or courtship than to do with the conditions under which animals carried or deposited larvae. These toads were also not old (captive bred winter 2011/12) and so the age of the toads may have contributed to lower fertility. The previous owner of this adult group anecdotally reported similar hatching success in the single breeding season under his care (S. Bogaerts pers. comm.).

A. obstetricans breed with rising temperatures in the summer, when toads and tadpoles are warm enough to be active and there is high enough productivity to support larval development, although lack of water in high

summer in some lowland areas in the south of its distribution may cause a reproductive hiatus in this species in the hottest months (Marquez, 2006; Masó & Pijoan, 2011). In captivity, replicating this temperature regime also led to reproduction. Although our animals originated from a different part of the range from where field data were collected, these data are still informative as to the microclimate preferences of this wide-ranging species and proved useful in successful captive breeding. However, reproductive success was not equal between populations (see above) and microclimate data from collection sites may have improved reproductive output in the groups. The field data suggest that toads are able to withstand, and actively choose to remain exposed to, temperatures exceeding those provided in captivity. In the field, toads only abandoned the focal refugia when temperature climbed consistently above 40°C and the substrate became increasingly dry. We chose to use a thermal gradient reaching lower temperatures to avoid inadvertently reaching critical thermal maximum for animals that, in captivity, are more limited in their ability to behaviourally regulate their immediate external, and therefore internal, environment.

Within the populations of animals included in this study, *A. obstetricans* seemed to require both a winter cooling period and a hot summer to stimulate breeding; those that were brumated but not provided with hot summer temperatures did not spawn, even though females developed eggs and stocking density and sex ratio remained identical between split populations. The use of an incandescent lamp over piles of broken tile to create a thermal gradient in vivaria replicates the type of habitat these toads are found in during summer months, such as rock piles, crevices in exposed grassy areas, and drystone walls. The thermal gradient produced in our vivaria was within the range recorded for wild toads during the breeding season; indeed, wild animals shelter in crevices with even higher temperatures and it is possible that even warmer conditions could be applied in captivity.

In the year prior to this study, the captive *A. obstetricans* were not overwintered at cool temperatures (reaching a low of only 12°C) and no animals reproduced although females were clearly gravid. Moreover, *A. obstetricans* did not breed when brumation period was 57 days (Lac d'Ansabere population; table 1) rather than 65 days (Lac de Lhurs and Lac d'Arlet populations). Given the lack of replicates this evidence is anecdotal, but there may be an effect of brumation period on successful gamete production as is the case in some other amphibians (e.g. Rastogi, 1976; Jorgensen, 1986; Laszlo, 1984; Paniagua *et al.*, 1990). Alternatively, differences between populations or random variation may explain this difference; further research with replicates may be of use here. There are no obvious environmental explanations for this variation in the collection sites (F. Claire, pers. comm.), but there are not sufficient data from adult refugia to formally investigate this. Temperate anurans, including *Alytes*, may evolve population specific reproductive thermal optima to cater for local environmental conditions (Llusia *et al.*, 2013a; b) and so subtle differences between the microclimates in respective collection sites may have driven different optimal temperatures for breeding in captivity. All groups had the potential to reproduce - all female toads developed obvious eggs in the abdomen during the autumn at temperatures of 11-12°C, but in the Lac d'Ansabere (57 day brumation) population, these had been completely resorbed and were no longer visible by 75 days post brumation in both animals with and without access to a basking lamp. It is unclear why the Lac de Lhurs population was less successful in carrying eggs to term than the Lac d'Arlet toads; all measured environmental parameters were identical, but again local adaptation may have meant that given parameters were less suitable for this population than for others. Neither *Alytes obstetricans* nor *A. cisternasii* are commonly maintained in captivity, but where they are *A. obstetricans* is rarely if ever bred in indoors enclosures in captivity, whereas *A. cisternasii* is frequently reproduced in vivaria. Most breeders of *A. obstetricans* only report success in outdoors enclosures and greenhouses (pers. obs.). Indeed, the experiments of Paul Kammerer in the 1920s raised controversy mainly due to his support of Lamarckian evolution, but to a lesser degree because of the readiness with which his *A. obstetricans* were reported to reproduce in captivity (Koestler, 1971). Keepers usually maintain this species without thermal gradients (pers. obs.) indoors and it is likely that the use of lamps to generate such gradients is necessary for successful captive reproduction in this species. However, further investigation with more replicates and the inclusion of additional localities of *A. obstetricans* from across its range would be useful in determining this.

Moreover, the work presented here for both species did not investigate all possible reproductive triggers and other cues that were not monitored may have been involved in stimulating reproduction, especially those that necessarily correlate with the provision of basking spots, such as humidity, which is inversely correlated with basking spots but positively associated with breeding periods for *A. cisternasii* in nature (Jiménez, 1984); in the present work, removal of basking spots in the winter may have led to an increase in humidity as well as a fall in temperature, prompting spawning. In any case, the toads clearly choose to use warm areas in the enclosure and as such basking spots may be important to provide to captive amphibians for thermoregulation.

This comparison underscores the importance of caution when applying the analogue species concept (Michaels *et al.*, 2014; Tapley *et al.*, 2015b), that is the use of a common relative of a rare species to develop captive husbandry protocols. Even closely related congeners may have very different requirements to stimulate

reproduction, but understanding the native climate and microhabitat selection can provide clues as to how to select appropriate analogues and to maintain amphibian species in captivity. It is likely that the patterns identified for *A. cisternasii* and *A. obstetricans* will extend to other *Alytes* species in captivity, according to whether their spawning period is defined mainly by hydroperiod or temperature in nature. Of course, such trends will be subject to adaptation to local population environmental conditions, but this general framework may be used to identify potential key reproductive triggers. *Alytes maurus*, although being distributed in North Africa, has a highland distribution and as such breeds in late Spring and early summer, much like *A. obstetricans*, of which it was, until recently considered a subspecies (Donaire-Barroso, 2006; Marquez *et al.*, 2011). It is likely that the provision of a long, cold winter period followed by the creation of a warm summer microclimate would stimulate breeding in this species. *Alytes dickhilleni*, although having a southern Spanish distribution, lives at a range of altitudes and breeds in more permanent water bodies (Bosch *et al.*, 2009b). Its breeding phenology is variable between sites and altitudes; at higher altitudes, usually eggs are deposited through the summer (Martinez-Solano *et al.*, 2003; Masó & Pijoan, 2011). At lower altitudes, where the climate may be milder and drier, this may occur through the winter and be more dependent on water availability (Malaga; D. Garcia-Alonso pers. comm.). In captivity, this species breeds readily in the late spring and summer when exposed to a seasonal temperature similar to that used for *A. cisternasii* and does not seem to require the hot basking sites apparently required for *A. obstetricans* (D. Garcia-Alonso pers. comm.)

Alytes muletensis is slightly unusual in that this species falls between both hydroperiod- and temperature-governed *Alytes* groups. *A. muletensis* naturally breeds in torrent pools and so in nature is restricted to breeding largely in May and June, the part of the hydroperiod when water is present, but when the water level is not so high that larvae are washed away (Serra *et al.*, 2009). However, this species also stops breeding at cool temperatures during the winter. This species reproduces throughout the spring, summer and autumn in captivity provided that temperatures are not below around 15°C and that water is present (Tonge & Bloxham, 1989; pers. obs.); success in brooding clutches to full term may be optimal between 23-25°C (pers. obs.).

Alytes cisternasii may spawn for less than a week in the field (Marquez, 1992), dependent on water availability, although at some sites the hydroperiod is longer and tadpoles are present for much of the winter (Jiménez, 1992). In captivity, our animals exhibited a relatively extended spawning period consistent with the presence of suitable breeding conditions. Like *A. muletensis*, it may be possible for *A. cisternasii* to reproduce year-round under cool moist conditions, but the long-term impact of reduced seasonality and lack of rest period on health and reproductive success are not fully resolved. Certainly, in other anurans, the lack of a rest period (albeit winter cooling) can lead to reduced adult size and reproductive output (Jorgensen, 1986; Reading, 2007).

These observations highlight the importance of understanding wild biology and phenology and replicating wild seasonality to stimulate breeding in captive amphibians, and potentially in choosing appropriate “analogue” species for the development of captive husbandry. In the case of *A. obstetricans* they also highlight the utility of even simple field data in informing captive husbandry.

ACKNOWLEDGEMENTS

We would like to thank Sergé Bogaerts for donating *Alytes cisternasii* to the ZSL London Zoo collection and for his advice on housing this species. We would also like to thank Dr. Frances Claire and Dr. Trenton Garner (ZSL Institute of Zoology) for providing our population of *A. obstetricans*. Finally, we are grateful to Steven Plested for his assistance in the husbandry of animals.

LITERATURE CITED

- Beja, P., Bosch, J., Tejedo, M., Lizana, M., Martínez-Solano, I., Salvador, A., García-París, M., Gil, E.R., Arntzen, J.W., Marquez, R., Paniagua, C.D. (2009). *Alytes cisternasii*. *The IUCN Red List of Threatened Species* 2009: e.T55266A11283060. Available from www.iucnredlist.org (accessed July 2015).
- Bosch, J., Beebee, T., Schmidt, B., Tejedo, M., Martínez-Solano, I., Salvador, A., García-París, M., Gil, E.R., Arntzen, J.W., Paniagua, C.D., Marquez, R. (2009a). *Alytes obstetricans*. *The IUCN Red List of Threatened Species* 2009: e.T55268A11283700. Available from www.iucnredlist.org (accessed July 2015).
- Bosch, J., Tejedo, M., Lizana, M., Martínez-Solano, I., Salvador, A., García-París, M., Recuero Gil, E., Arntzen, J.W., Marquez, R., Paniagua, C.D., Podloucky, R. (2009b). *Alytes dickhilleni*. *The IUCN Red List of Threatened Species* 2009: e.T979A13099604. Available from www.iucnredlist.org (accessed July 2015).
- Böll, S., Linsenmair, K.E. (1998). Size-dependent male reproductive success and size-assortative mating in the

- midwife toad *Alytes obstetricans*. *Amphibia-Reptilia*, 19: 75-89.
- Bosch, J., Márquez R. (1996). Discriminant functions for sex identification in two midwife toads (*Alytes obstetricans* and *A. cisternasii*). *Herpetological Journal*, 6: 105-109.
- Bosch, J., González-Miras, E. (eds.) (2012). *Seguimiento de Alytes dickhilleni: Informe final*. Monografías SARE. Asociación Herpetológica Española. Ministerio de Agricultura, Alimentación y Medio Ambiente, Madrid [Spanish].
- Buchholz, S. (1989). *Untersuchungen zur Fortpflanzungsbiologie und Populationsdynamik einer Freilandpopulation von Alytes obstetricans (Amphibia, Anura, Discoglossidae)*. Thesis. University of Würzburg, Würzburg [German].
- Crespo, E.G. (1979). *Contribuição para o conhecimento da biologia das espécies Ibéricas de Alytes, Alytes obstetricans boscai Lataste, 1879 e A. cisternasii Bosca, 1879 (Amphibia, Salientia): a problemática da especiação de A. cisternasii*. PhD thesis. University of Lisbon, Lisbon [Portuguese].
- Donaire-Barroso, D., El Mouden, E.H., Slimani, T., de la Vega, J.P.G. (2006). On the meridional distribution of *Alytes maurus* Pasteur and Bons, 1962 (Amphibia, Discoglossidae). *Herpetological Bulletin*, 96: 12.
- 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.
- Galan, P., Vences, M., Glaw, F., Fernandez-Arias, B., Garcia-Paris, M. (1990). Beobachtungen zur Biologie von *Alytes obstetricans* in Nordwestberien. *Herpetofauna*, 12: 17-24 [German with English summary].
- Gascon, C., Collins, J.P., Moore, R. D., Church, D. R., McKay, J. E., Mendelson, J.R. III (eds.). (2007). *Amphibian Conservation Action Plan*. IUCN/SSC Amphibian Specialist Group. Gland, Switzerland and Cambridge, UK.
- Jørgensen, C.B. (1984). Relations between brumation and ovarian functions in a temperate zone frog, *Rana temporaria*. *Acta Zoologica*, 65: 239-247.
- Jørgensen, C.B. (1986). External and internal control of patterns of feeding, growth and gonadal function in a temperate zone anuran, the toad *Bufo bufo*. *Journal of Zoology*, 210: 211-241.
- Jiménez, A.J.R. (1984). Fenología del sapo partero Iberico (*Alytes cisternasii* Bosca 1879). *Alytes*, España, 2: 9-23 [Spanish with French summary].
- Jiménez, A.J.R. (1992). Fenología de una comunidad de anfibios asociada a cursos fluviales temporales. *Acta Vertebrata*, 15: 29-43 [Spanish with English summary].
- Koestler, A. (1971). *The case of the midwife toad*. Hutchinson, London.
- Laszlo, J. (1984). Further notes on reproductive patterns of amphibians and reptiles in relation to captive breeding. *International Zoo Yearbook*, 23: 166-174.
- Llusia, D., Márquez, R., Beltrán, J.F., Moreira, C., Do Amaral, J.P. (2013). Environmental and social determinants of anuran lekking behavior: intraspecific variation in populations at thermal extremes. *Behavioral Ecology and Sociobiology*, 67: 493-511.
- Llusia, D., Márquez, R., Beltrán, J.F., Benitez, M., Do Amaral, J.P. (2013). Calling behaviour under climate change: geographical and seasonal variation of calling temperatures in ectotherms. *Global Change Biology*, 19: 2655-2674.
- Malkmus, R. (1983). Zur Fortpflanzungsbiologie von *A. cisternasii*. *Herpetofauna*, 5: 30-34 [German].
- Márquez, R. (1992). Terrestrial paternal care and short breeding seasons: reproductive phenology of the midwife toads *Alytes obstetricans* and *A. cisternasii*. *Ecography*, 15: 279-288.
- Marquez, R., Verrell, P. (1990). The courtship and mating of the iberian midwife toad *Alytes cisternasii* (Amphibia: Anura: Discoglossidae). *Journal of Zoology*, London, 225: 125-139.
- Márquez, R.. (1993). Male reproductive success in two midwife toads, *Alytes obstetricans* and *A. cisternasii*. *Behavioral Ecology and Sociobiology*, 32: 283-291.
- Márquez, R.. (1996). Egg mass and size of tadpoles at hatching in the midwife toads *Alytes obstetricans* and *Alytes cisternasii*: implications for female choice. *Copeia*, 1996: 824-831.
- Márquez, R., Esteban, M., Castanet, J. (1997). Sexual size dimorphism and age in the midwife toads *Alytes obstetricans* and *A. cisternasii*. *Journal of Herpetology*, 31: 52-59
- Márquez, R., Francisco Beltrán, J., Slimani, T., Radi, M., Llusia, D., El Mouden, H. (2011). Description of the advertisement call of the moroccan midwife toad (*Alytes maurus* Pasteur & Bons, 1962). *Alytes*, 27: 142-150.

- Martinez-Solano, I., Paris, M., Izquierdo, E., Garcia-Paris, M. (2003). Larval growth plasticity in wild populations of the Betic midwife toad *Alytes dickhilleni* (Anura: Discoglossidea). *Herpetological Journal*, 13: 89-94.
- Masó, A., Pijoan, M. (2011). *Anfibios y reptiles de la Península Ibérica, Baleares y Canarias*. Nuevas guías de campo, Ediciones Omega, Barcelona [Spanish].
- Michaels, C.J., Antwis, R.E., Preziosi, R.F. (2015). Impacts of UVB provision and dietary calcium content on serum vitamin D3, growth rates, skeletal structure and coloration in captive oriental fire-bellied toads (*Bombina orientalis*). *Journal of Animal Physiology and Animal Nutrition*, 99: 391-403.
- Michaels, C.J., Gini, B.F., Preziosi, R.F. (2014). The importance of natural history and species-specific approaches in amphibian ex-situ conservation. *The Herpetological Journal*, 24: 135-145.
- Michaels, C.J., Preziosi, R.F. (2013) Basking behaviour and ultraviolet B radiation exposure in a wild population of *Pelophylax lessonae* in northern Italy. *Herpetological Bulletin*, 124: 1-8.
- Paniagua, R., Fraile, B., Saez, F.J. (1990). Effects of photoperiod and temperature on testicular function in amphibians. *Histology and Histopathology* 5: 365-78.
- Rastogi, R. K. (1976). Seasonal cycle in anuran (Amphibia) testis: The endocrine and environmental controls. *Italian Journal of Zoology*, 43: 151-172.
- Reading, C.J., Clarke, T. (1988) Multiple clutches, egg mortality and mate choice in the midwife toad, *Alytes obstetricans*. *Amphibia-Reptilia*, 9: 357-364.
- Reading, C.J. (2007). Linking global warming to amphibian declines through its effects on female body condition and survivorship. *Oecologia*, 151: 125-131.
- Serra, J.M., Griffiths, R., Bosch, J., Beebee, T., Schmidt, B., Tejedo, M., Lizana, M., Martínez-Solano, I., Salvador, A., García-Paris, M., Gil, E.R., Arntzen, J.W. (2009). *Alytes muletensis*. *The IUCN Red List of Threatened Species* 2009: e.T977A13099129. Available from www.iucnredlist.org (accessed July 2015).
- Tapley, B., Rendle, M., Baines, F.M., Goetz, M., Bradfield, K.S., Rood, D., Routh, A. (2015a). Meeting ultraviolet B radiation requirements of amphibians in captivity: A case study with mountain chicken frogs (*Leptodactylus fallax*) and general recommendations for pre-release health screening. *Zoo Biology*, 4: 46-52.
- Tapley, B., Bradfield, K., Michaels, C.J., Bungard, M. (2015b). Amphibians and conservation breeding programmes: do all threatened amphibians belong on the ark? *Biodiversity and Conservation*, 24: 2625-2646.
- Tonge, S., Bloxam, Q. (1989). Breeding the Mallorcan midwife toad *Alytes muletensis* in captivity. *International Zoo Yearbook*, 28: 45-53.
- Verschooren, E., Brown, R.K., Vercammen, F., Pereboom, J. (2011). Ultraviolet B radiation (UV-B) and the growth and skeletal development of the Amazonian milk frog (*Trachycephalus resinifictrix*) from metamorphosis. *Journal of Physiology and Pathophysiology*, 2: 34-42.
- Wells, E., Garcia-Alonso, D., Rosa, G.M., Garcia, G., Tapley, B. (2015). *Amphibian Taxon Advisory Group best practice guidelines for midwife toads (Alytes spp.)*, version 1. Amphibian Taxon Advisory Group, EAZA. Available from www.eaza.net/assets/Uploads/CCC/2015-Midwife-toads-EAZA-Best-Practice-Guidelines-Approved.doc.pdf
- World Weather Online (2015). Algarve monthly climate average, Portugal. Available from www.worldweatheronline.com/Algarve-weather-averages/PT.aspx (accessed July 2015).
- Zippel, K., Lacy, R., Byers, O. (2006). *CBSG/WAZA amphibian ex situ conservation planning workshop*. Final report. IUCN/SSC Conservation Breeding Specialist Group, Apple Valley, MN.