

Amphibian Taxon Advisory Group Best Practice Guidelines for Midwife toads (*Alytes* sp.)

Version 1



Authors: Elizabeth Wells, David Garcia-Alonso, Gonçalo M. Rosa, Gerardo Garcia & Benjamin Tapley

ATAG Chair: Gerardo Garcia, Chester Zoo, United Kingdom, CH2 1LH. ATAG Vice-chairs: Warren Spencer Plantage Kerklaan 38-40, 1018 CZ Amsterdam, Netherlands. Olivier Marquis, Parc Zoologique de Paris, 51 avenue de Saint-Maurice, 75012 Paris, France.

Published: 2015

EAZA Best Practice Guidelines disclaimer

Copyright (2015) by EAZA Executive Office, Amsterdam. All rights reserved. No part of this publication may be reproduced in hard copy, machine-readable or other forms without advance written permission from the European Association of Zoos and Aquaria (EAZA). Members of the European Association of Zoos and Aquaria (EAZA) may copy this information for their own use as needed.

The information contained in these EAZA Best Practice Guidelines has been obtained from numerous sources believed to be reliable. EAZA and the EAZA Amphibian TAG make a diligent effort to provide a complete and accurate representation of the data in its reports, publications, and services. However, EAZA does not guarantee the accuracy, adequacy, or completeness of any information. EAZA disclaims all liability for errors or omissions that may exist and shall not be liable for any incidental, consequential, or other damages (whether resulting from negligence or otherwise) including, without limitation, exemplary damages or lost profits arising out of or in connection with the use of this publication.

Because the technical information provided in the EAZA Best Practice Guidelines can easily be misread or misinterpreted unless properly analysed, EAZA strongly recommends that users of this information consult with the editors in all matters related to data analysis and interpretation.

Preamble

Right from the very beginning it has been the concern of EAZA and the EEPs to encourage and promote the highest possible standards for husbandry of zoo and aquarium animals. For this reason, quite early on, EAZA developed the “Minimum Standards for the Accommodation and Care of Animals in Zoos and Aquaria”. These standards lay down general principles of animal keeping, to which the members of EAZA feel themselves committed. Above and beyond this, some countries have defined regulatory minimum standards for the keeping of individual species regarding the size and furnishings of enclosures etc., which, according to the opinion of authors, should definitely be fulfilled before allowing such animals to be kept within the area of the jurisdiction of those countries. These minimum standards are intended to determine the borderline of acceptable animal welfare. It is not permitted to fall short of these standards. How difficult it is to determine the standards, however, can be seen in the fact that minimum standards vary from country to country.

Above and beyond this, specialists of the EEPs and TAGs have undertaken the considerable task of laying down guidelines for keeping individual animal species. Whilst some aspects of husbandry reported in the guidelines will define minimum standards, in general, these guidelines are not to be understood as minimum requirements; they represent best practice. As such the EAZA Best Practice Guidelines for keeping animals intend rather to describe the desirable design of enclosures and prerequisites for animal keeping that are, according to the present state of knowledge, considered as being optimal for each species. They intend above all to indicate how enclosures should be designed and what conditions should be fulfilled for the optimal care of individual species.

Introduction

The information in this Best Practice Guideline has come from a variety of sources including an extensive literature review, the experience of the authors and others in the captive husbandry of *Alytes* species as well as direct observations of the species in the field.

Amphibian husbandry is a rapidly evolving field and there are many aspects that require further research. Water parameters and UVB exposure in the field require documentation. It has only been possible to recommend water parameters and UV provision based on captive husbandry experience and a more evidence based approach utilising parameters from the field should be developed in order to make evidence based husbandry recommendations.

Captive diets for both larval and post metamorphic amphibians are likely to differ from diets consumed by larval and post metamorphic amphibians in the field. Replicating the wild diet in captivity will likely be precluded by the limited number of invertebrate species that can be reared on scale required for them to form viable live food colonies.

Key husbandry points

1. The provision of appropriate UV, temperature and humidity gradients within the enclosure (this will vary depending on the species and the time of year).
2. Monitoring egg carrying males. Entanglement of the egg masses around the hind legs of male specimens can result in an annular constriction around the distal aspect of the tibial region. This can cut through the soft tissues of the leg and /or result in the obstruction of the blood flow. If not detected early and treated, the foot swells up and may eventually become necrotic.
3. Monitoring and management of water quality.

These guidelines have been reviewed and approved by the Amphibian TAG members.

Contents

Section 1. BIOLOGY AND FIELD DATA.....	5
1.1. Taxonomy.....	5
1.2. Morphology.....	6
1.2.1 Colouration.....	7
1.2.2 Description.....	7
1.2.3 Longevity.....	9
1.3 Conservation status/ Zoogeography/Ecology.....	9
1.3.1 Distribution.....	9
1.3.2 Habitat	12
1.3.3 Conservation status and population	15
1.4 Diet and feeding behaviour.....	17
1.4.1 Feeding behaviour.....	17
1.5 Reproduction.....	18
1.5.1 Developmental stages to sexual maturity.....	18
1.5.2 Age of sexual maturity.....	19
1.5.3 Gestation period / Incubation	20
1.5.4 Clutch size.....	21
1.5.5 Hatching details an season.....	21
1.6 Behaviour.....	22
1.6.1 Activity.....	22
1.6.2 Locomotion.....	23
1.6.3 Predation.....	24
1.6.4 Social behaviour.....	25
1.6.5 Sexual behaviour.....	25
Section 2. MANAGEMENT IN ZOOS AND AQUARIA.....	27
2.1 Enclosure.....	27
2.1.2 Substrate.....	27
2.1.3 Furnishing and maintenance.....	27
2.1.4 Environment.....	29
2.1.5 Seasonality.....	31
2.1.6 UV, Light and heat.....	30
2.1.7 Dimensions and stocking density.....	31
2.2 Feeding and water.....	32
2.2.1 Basic diet.....	32
2.2.2 Special dietary requirements.....	33
2.2.3 Method of feeding.....	33
2.2.4 Water requirements.....	33
2.3 Social structure.....	36
2.3.1 Changing social structure.....	36
2.3.2 Sharing enclosure with other species.....	37
2.4 Breeding.....	37
2.4.1 Mating.....	37
2.4.2 Pregnancy / egg laying / incubation.....	37
2.4.3 Use of hormones.....	38
2.4.4 Birth / hatchling.....	38
2.4.5 Larval development.....	39
2.4.6 Population management.....	39
2.5 Handling.....	39
2.5.1 Individual identification and handling.....	39
2.5.2 General handling.....	41
2.5.3 Transportation.....	41
2.5.4 Safety.....	42
2.6 Specific problems.....	43
2.7 Recommended research.....	43
Acknowledgements.....	44
References.....	45

SECTION 1: BIOLOGY AND FIELD DATA

BIOLOGY

1.1 Taxonomy

ORDER: Anura

FAMILY: Alytidae

GENUS: *Alytes* Wagler, 1830

SPECIES: *A. cisternasii* Boscá, 1979; *A. dickhilleni* Arntzen & García-París, 1995; *A. maurus* Pasteur & Bons, 1962; *A. muletensis* (Sanchíz & Adrover, 1979); *A. obstetricans* (Laurenti, 1768)

COMMON NAMES

Alytes spp. Midwife toad

Alytes cisternasii: Iberian midwife toad

Alytes dickhilleni: Betic midwife toad

Alytes maurus: Moroccan midwife toad; Crapaud Accoucheur

Alytes muletensis: Mallorca midwife toad; Ferreret

Alytes obstetricans: Common midwife toad

Subspecies:

Alytes obstetricans:

Alytes obstetricans almogavarii Arntzen & García-París, 1995

Alytes obstetricans boscai Lataste, 1849

Alytes obstetricans obstetricans Laurenti, 1968

Alytes obstetricans pertinax García-París & Martínez-Solano, 2001

1.2 Morphology

SNOUT-VENT LENGTH

Species	Mean adult SVL (mm)	Newly metamorphosed toadlets (mm)	Newly hatched larvae (length mm)	Larvae total (length mm)	Reference
<i>Alytes cisternasii</i>	Mean: 39 Male: 36 Female: 42	24	13	70	Noellert & Noellert, 1992
<i>Alytes dickhilleni</i>	Mean: Sierra de Cazorla: mean= 41 mm Sierra de Baza: mean 49 mm; range= 41-56.5 mm	15-21	13	74	Arntzen & Garcia-Paris, 1995; Márquez & Bosch, 1996; Gonzalez-Miras, unpublished data; D. García-Alonso & A. Escolano, Unpublished data; M. Benitez, unpublished data
<i>Alytes maurus</i>	Mean: 43.5 Male: NR Female: 32	NR	NR	40	Pasteur & Bons, 1962; Noellert & Noellert, 1992
<i>Alytes muletensis</i>	Mean: 36.4 Male: 34.7 Female: 38	20.5	18	7688	Tonge & Bloxam, 1989; Noellert & Noellert, 1992; Olivier, 2004
<i>Alytes obstetricans</i>	Mean: 30.27-38.24 Male: 46.0 Female: 51	21.0	15	70, over wintered larvae can attain a total length of 110mm	Engelmann, 1985; Noellert & Noellert 1992, G.M. Rosa, Pers. comm; F. Clare, Pers. comm.

Table 1 Snout-vent length data for *Alytes* species. NR= Not Recorded. Adult *Alytes muletensis* measurements taken from individuals at Durrell; Adult *Alytes obstetricans* measurements taken from captive individuals at ZSL London Zoo, origin: Lhurs, France.

EGG SIZE

Species	Diameter when freshly laid (mm)	Maximum diameter (mm)	References
<i>Alytes cisternasii</i>	2.6-3.5	4.4	Marquez & Verrell, 1990
<i>Alytes dickhilleni</i>	NR	NR	
<i>Alytes maurus</i>	NR	NR	
<i>Alytes muletensis</i>	NR	7	Noellert & Noellert, 1992
<i>Alytes obstetricans</i>	NR	NR	

Table 2 Egg size data for *Alytes* species.

WEIGHT

Species	Adult (g)	Newly metamorphosed toadlet (g)	References
<i>Alytes cisternasii</i>	NR	NR	
<i>Alytes dickhilleni</i>	6.03	0.5-0.9	D. Garcia-Alonso, Unpublished data
<i>Alytes maurus</i>	NR	NR	
<i>Alytes muletensis</i>	10.2	0.65	Tonge & Bloxam, 1989
<i>Alytes obstetricans</i>	3.74-6.8	1.1.5	F. Clare, Pers. comm.

Table 3 Weight measurements for *Alytes* species. Adult *A. muletensis* measurements taken from individuals at Durrell; Adult *A. obstetricans* measurements taken from captive individuals at ZSL London Zoo, origin: Lhurs, France; Measurements from *A. dickhilleni* from Bioparc Fuengirola and the field.

1.2.1 COLOURATION

A. cisternasii: Colouration brown; pattern of dark spots and mostly red warts; underside colouration dirty white; often a light colour band between the eyes (Noellert & Noellert, 1992; Figs. 1 and 2).

A. dickhilleni: Colouration black-grey; pattern of grey, black and light-coloured dots; commonly a patch of grey between the eyes (Van der Meijden & Cavagnaro, 1999; Fig. 3).

A. maurus: Colouration varies for the dorsal region; pattern of small black, brown or olive green spots; venter colouration whitish; often throat and chest pattern of grey spots (Noellert & Noellert, 1992; Fig. 4).

A. muletensis: Colouration yellow or cream; pattern of varied dark green or brown markings on the head, limbs and dorsum; underside colouration white; sometimes a black triangle on the head behind the eyes (Tonge & Bloxam, 1989; (Noellert & Noellert 1992; Fig. 5).

A. obstetricans: Colouration varies: pattern of small black, green, brown or olive spots; underside colouration dirty white; often a chest and throat pattern of grey spots (Noellert & Noellert, 1992; Fig. 6).

1.2.2 DESCRIPTION

The five species in the genus *Alytes* are described as small stocky frogs with a relatively large head and large eyes with a vertical slit-shaped pupil (Fig 1-6). In *A. cisternasii*, *A. maurus* and *A. obstetricans* the skin is warty, whereas that of *A. muletensis* is relatively smooth. The parotid glands are reduced in *A. muletensis*.

A. cisternasii, *A. dickhilleni*, *A. muletensis* and *A. obstetricans* have three metacarpal tubercles. *A. cisternasii*, *A. maurus* and *A. obstetricans* exhibit a row of red warts from the tympanum which extends to the hind limb insertion in *A. maurus* and to the loin in *A. cisternasii* and *A. obstetricans*. Sexual dimorphism is exhibited in *A. cisternasii*, *A. maurus* and *A. obstetricans*, and *A. muletensis*. In the former three species males and females are differentiated by subtle differences between the distances between nostrils, distance between the anterior end of the middle metacarpal tubercle and tip of the third finger, and distance between the elbow and tip of the third finger (Pasteur & Bons, 1962; Arntzen & Garcia-Paris, 1995; Bosch & Marquez, 1996; Noellert & Noellert 1992; Van der Meijden, 1999a; Dieterich, 2014; Van der Meijden, 1999b).



Fig. 1. (Top left) Adult *A. cisternasii* (S. Bogaerts); Fig. 2. (Top right) Juvenile *A. cisternasii* (S. Bogaerts); Fig. 3. (Middle left) *A. dickhilleni* (Wouter Beukema, 2008); Fig. 4. (Middle right) Juvenile *A. maurus* in the Rif Mountains of Morocco (R. Marquez, 2006); Fig. 5. (Bottom left) Female *A. muletensis* in Mallorca (B. Tapley); Fig. 6. Captive adult *A. obstetricans* (B. Tapley).

Adult *A. maurus* and *A. obstetricans* are very hard to distinguish from one another based on morphology; it is easier to tell them apart in larval form, as they possess several distinctive traits: *A. maurus* larvae possess a pigmented network of chromatophores that follow a loose irregular grid structure, as opposed to a more regular grid on *A. obstetricans*. *A. maurus* larvae commonly have a dark trisegmented border on the lower jaw that is absent in *A. obstetricans*. Tooth morphology differs in the two species: in *A. maurus* the upper anterior tooth row comprises two rows of uniform density, and every other row contains at least one more row than in *A. obstetricans* (Pasteur & Bons, 1962; Dietterich, 2014; Van der Meijden, 1999b).

1.2.3 Longevity

In the wild *A. muletensis* has a maximum longevity of 18 years (Pinya & Pérez-Mellado, 2013). Bush (1993) estimates longevity at 7 years for captive populations of *A. muletensis*. Longevity estimates from wild populations are 6 years for *A. cisternasii* (Márquez et al., 1997); 9 years for *A. dickhilleni* (González-Miras et al., 2012) and 7 years for *A. obstetricans* (Böllet et al., 1997; Márquez et al., 1997).

Information for the longevity of *A. maurus* is currently unpublished.

FIELD DATA

1.3 Conservation status/ Zoogeography/Ecology

1.3.1 DISTRIBUTION

A. cisternasii is native to Spain and Portugal, and its range is restricted to Southern and Eastern Portugal, and Western and Central Spain, between 100m and 1,300m asl (Beja et al., 2009; Fig. 7).



Fig. 7. Distribution map for *A. cisternasii* from the IUCN Red List (Beja et al., 2009).

A. dickhilleni is native to Spain, and found only in mountains in the south east, at altitudes of 79 m to 2,510 m asl (Bosch et al., 2009b; Bosch & Gonzalez-Miras, 2012; Fig. 8).



Fig. 8. Distribution map of *A. dickhilleni* from the IUCN Red List (Bosch et al., 2009b).

A. maurus is native to Morocco and the North African Spanish enclave of Ceuta, but is found only in the Western and Central Rif Mountains and Middle Atlas Mountains of Morocco, from 200 m to 2,050 m asl. It is known to occur in 30 localities with an extent of occurrence less than 5000 km² in the wettest areas of the Rif and Middle Atlas of Morocco (Donaire-Barroso et al., 2006; Donaire-Barroso et al., 2009; Dietterich, 2014; Fig. 9).



Fig. 9. Distribution map for *A. maurus* from the IUCN Red List (Donaire-Barroso et al., 2009).

A. muletensis is endemic to the island of Mallorca, where its range is restricted to the Sierra Tramuntana in the North of the Island, between 10m and 850 m asl in an increasing area of 10 km² (Serra et al., 2009). *A. muletensis* is currently limited to less than 20 sites, 6 of which are populations established from reintroductions (Kraaijeveld-Smit et al., 2005). The range restriction of the wild population of *A. muletensis* may correspond with the ranges of predatory snakes, i.e. where snakes are not found or are few in number (Schley & Griffiths, 1997; Fig. 10).

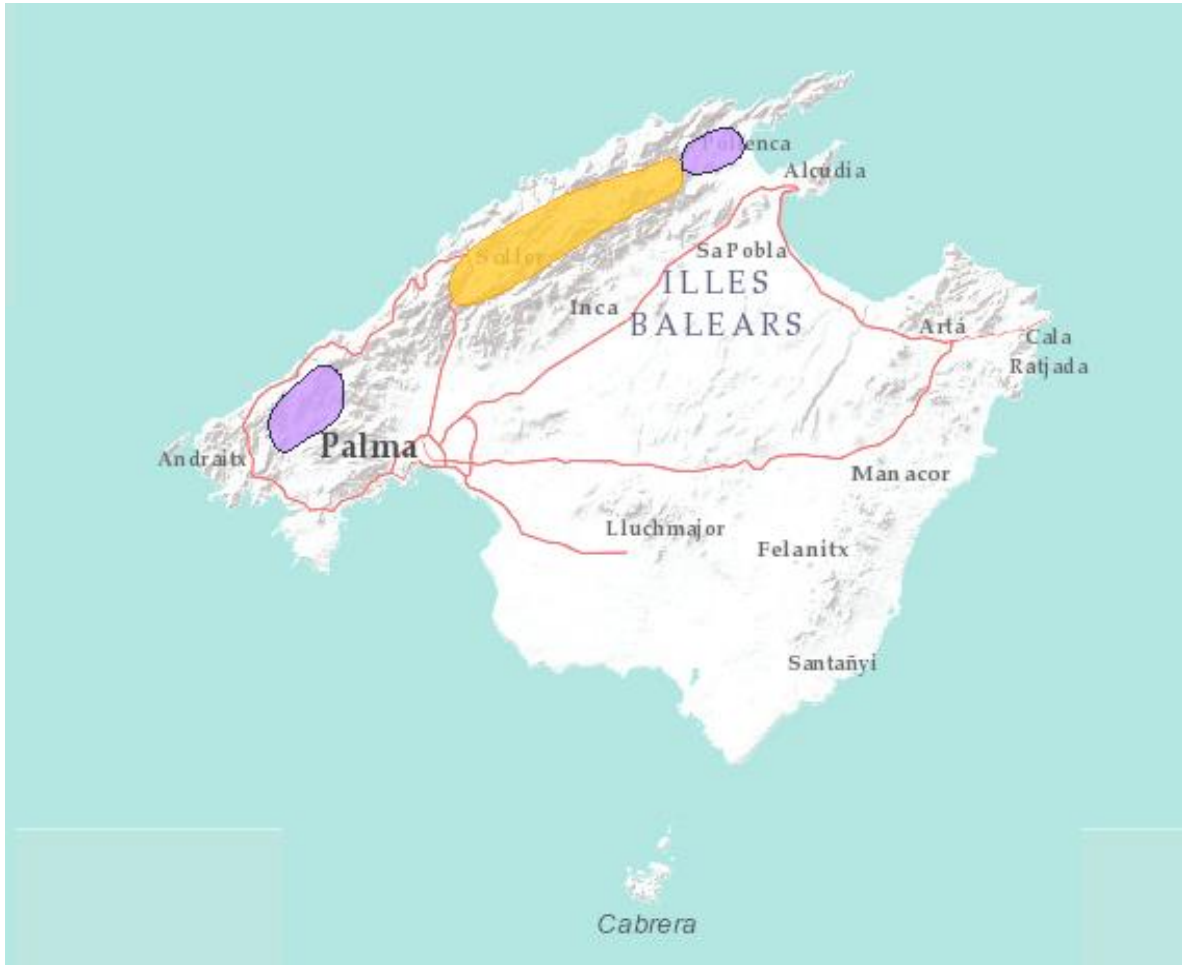


Fig. 10. Distribution map for *A. muletensis* on the island of Mallorca, from IUCN Red List (Serra et al., 2009); orange (extant), purple (reintroduced).

A. obstetricans is native to Belgium; France; Germany; Luxembourg; Netherlands; Portugal; Spain; Switzerland, and has been introduced to the United Kingdom. It ranges from Northern parts of Portugal and Spain, through France to Southern Belgium, extreme South-eastern Netherlands, Luxembourg, western and North-central Germany, and western and Northern Switzerland. There are several established populations in the UK (Bedfordshire, Yorkshire, Northamptonshire, Hampshire, Devon and South-West London; RAUK, 2014). Its range extends from sea level to 2,400 m asl in the Pyrenees (Bosch et al., 2009a). The subspecies *A. o. almogavarii* is found in the Eastern Pyrenees and Northeast Spain; *A. o. boscai* range covers central and northern Portugal and north-west Spain; *A. o. obstetricans* occupies the range extent of this species throughout Europe; *A. o. pertinaxi* can be found along the Spanish Mediterranean coast up to the Ebro River (Barios et al., 2012; Fig. 11).



Fig. 11. Distribution map for *A. obstetricans* from IUCN Red List (Bosch et al., 2009a); orange (extant), red (extinct).

1.3.2 HABITAT

All *Alytes* species utilise both terrestrial and freshwater habitats. *A. cisternasii*, *A. dickhilleni* and *A. maurus* are associated with oak vegetation, although not exclusively. In all species breeding and larval development take place in streams, although *A. cisternasii*, *A. dickhilleni*, *A. maurus* and *A. muletensis* utilise mountain streams, whereas *A. obstetricans* lay eggs in slow-moving or stagnant water (Beja et al., 2009; Bosch et al., 2009a; Bosch et al., 2009b; Donaire-Barroso et al., 2009; Serra et al., 2009).

A. cisternasii is mostly associated with open and often sandy areas and is not considered very adaptable (Beja et al., 2009; Fig. 12). Specimens, including egg-carrying males, bury into the substrate backwards utilising the tubercles on their hind feet.



Fig. 12. The habitats of *A. cisternasii* (S. Bogaerts).

A. dickhilleni occurs in the arid South-east of the Iberian peninsula, and it is associated with open rocky areas on calciferous substrate and is also present in pine and oak forests. The species is closely associated with human settlement and appears to show a preference for mountainous areas, where traditional agricultural practices allow the persistence of such habitat (Egea-Serrano et al., 2006). The habitat of this species is characterised by unpredictable stresses on the water balance, and as the permanence of water sources has been shown to be an important factor in breeding habitat selection, this has led this species to utilise, man-made water sources. Approximately 80% of *A. dickhilleni* populations are associated with man-made water sources and only 20% to natural water sources, this is probably due to the degradation of their natural habitats (Bosch & Gonzalez-Miras, 2012). Specimens will select the terrestrial macrohabitat first and breed in available water bodies (Egea-Serrano et al., 2006). Adults spend time in rock fissures and on stones next to water sources, and breeding can take place in man-made reservoirs (Bosch et al., 2009b).

A. maurus inhabits humid areas of montane karst and escarpment, and is associated with scrub, cork oak groves and orchards. For cover, adults utilise rock fissures or under stones close to permanent streams, pools and other water bodies. Some breeding ponds are also inhabited by introduced predatory fish species *Gambusia holbrooki* (Donaire-Barroso et al., 2009).

The current distributional range of *A. muletensis* is positively correlated with steep slopes, and individuals can be found exclusively in streams and rock crevices in Limestone Mountains (Fig. 13). They utilise semi-permanent pools and streams dependant on the season, as well as man-made reservoirs such as cattle troughs and rain tanks. In their natural habitat water temperatures can range from 9°C to 22°C (Van der Meijden, 1999c). They do not tolerate serious habitat degradation. The distribution of predators of this species is negatively associated with elevation, and reproductive success of this species is positively associated with elevation. Sympatric species of note include the introduced predatory Viperine Snake (*Natrix maura*), and Perez's Frog (*Pelophylax perezii*) with which it competes for resources (Moore et al., 2004; Serra et al., 2009).



Fig. 13. *A. muletensis* larvae in a native pool in their home range of the Sierra Tramuntana Mountains in Mallorca (Benjamin Tapley).

A. obstetricans is found in terrestrial habitats including temperate forests, semi-arid areas, walls, embankments, and slopes with small stones and sparse vegetation. They are not often found in xeric sites due to the need for reliable bodies of water for breeding (Garcia-Paris, 1985). They utilise slow-

moving rivers and pools in their natural habitat, and gravel and clay pits in built-up environments, for breeding. They can occur in modified habitat such as agricultural land (Bosch et al., 2009a).

1.3.3 CONSERVATION STATUS AND POPULATION

A. cisternasii IUCN Red List Status: Near Threatened, close to qualifying as Vulnerable; the species is undergoing population declines, although they are thought to be less than 30%, and is locally abundant within suitable habitat (Beja et al., 2009). Populations are threatened by introduced fish and the Louisiana Crayfish (*Procambarus clarkii*), which are predators of their larvae (Cruz et al., 2008; Beja et al., 2009). Other threats include anthropogenic causes of habitat loss, both aquatic and terrestrial, through forest clearance, pollution, canal construction, dams and urbanisation. There is a potential future threat from the disease chytridiomycosis which is already known to have had negative impacts on populations of *A. obstetricans* in Spain and *A. muletensis* in Mallorca. *A. cisternasii* is protected under the Bern convention and EU Habitats Directive, and also under national legislation in Spain, as it occurs in the Cabañeros and Doñana National Parks (Beja et al., 2009).

A. dickhilleni IUCN Red List status: Vulnerable; subpopulations are declining and occupy less than 2,000 km². Populations are severely fragmented, with ranges contracting, and the species is becoming rare in drier areas. Loss of breeding habitats is a major problem, either through depletion of natural water sources for agriculture, or abandonment of traditional reservoirs such as cattle troughs due to modernisation. In 2011 it was discovered that *A. dickhilleni* was affected by *Batrachochytrium dendrobatidis* (*Bd*), the causative agent of amphibian chytridiomycosis, and the following year some populations had already experienced decline (Bosch et al., 2013). The species is protected under the Bern Convention as part of *A. obstetricans*, and it falls within the Parque Nacional Sierra Morena, Parque Nacional de Sierra Nevada, and the Natural Park of Cazorla, Segura y las Villas. There are current projects in Castilla-La Mancha, Andalusia, to restore breeding habitat (Bosch et al., 2009b).

A. maurus IUCN Red List status: Vulnerable, down-listed from Near Threatened in 2004; populations of this species appear stable and are locally common in suitable habitat, but they are fragmented and occur over less than 5000 km². Threats to this species are generally localised, and include the introduction of predatory fish (*Gambusia holbrooki*) to breeding ponds, and domestic water pollution in Chauen. Some populations do occur within protected areas, but the species is not assumed to be severely threatened (Donaire-Barroso et al., 2009; EDGEb, 2014).

A. muletensis IUCN Red List status: Vulnerable, up-listed from Endangered in 1990 to Critically Endangered in 1996, and down-listed to Vulnerable in 2004; it faced near extinction before recovery efforts and the population is now increasing, if at a slow pace, and currently stands at around 500-1,500 adult pairs in 25 mostly isolated populations (Serra et al., 2009). An annual larvae count found over 30,000 larvae in 2004, the highest on record. The species has received conservation attention in the form of reintroduction programs, but its range extent is still relatively small being less than 20 km², and it is known to occur in less than 5 locations; it is expected that populations of the species are unlikely to establish themselves in new areas without continued reintroduction efforts (Serra et al., 2009). Threats include predation of toads and larvae from introduced Viperine Snake (*Natrix maura*), competition for resources from Perez's Frog (*Pelophylax perezii*), and anthropogenic threats

from tourism and desiccation of breeding sights through water extraction and re-direction (Serra et al., 2009; EDGEc, 2014). Removal of snakes from the toads' territory has been identified as an important conservation measure, because whilst they have evolved some predator avoidance mechanisms in response to *N. maura*, the snakes may still be the cause of severe population declines (Schley & Griffiths, 1997). Disease is also a threat to populations of this species; *Bd* is already known to have infected wild populations on Mallorca as a result of a reintroduction program, which released infected individuals. These animals became infected with *Bd* in *ex situ* captive facilities (Walker et al., 2008). *A. muletensis* is protected under the Bern Convention and the EU Habitats Directive, occurs on national and sub-national Red Data Books, and is also present in the Tramuntana mountains protected areas; the capture or killing of this species is forbidden under regional law (Serra et al., 2009; EDGEc, 2014). Reintroduction programs coordinated by Durrell Wildlife Conservation Trust (Durrell) and the Balearic Government were carried out for several years and with great success, having established at least 10 populations on the Island, although these were stopped in 2004 after the discovery of disease in reintroduced populations (Serra et al., 2009; EDGEc). The species faces potentially dramatic declines if not closely monitored, and plans for a new reintroduction program are underway. There is also a program underway to remove *N. maura* from the range of *A. muletensis* (Serra et al., 2009).

A. obstetricans Red List Status: Least Concern; populations of this species are large, widely distributed across Europe and have broad habitat tolerances (Bosch et al., 2009a). Although still common across its range, it has been undergoing some population declines for a number of reasons. Loss of suitable habitat, including breeding sites, across its range, is leading to population fragmentation and possible isolation particularly in Spain and Portugal, as a result of agricultural development. There is also a threat to some populations from introduced predatory fishes such as the Pumpkin Seed fish *Lepomis gibbosus*, and salmonids, to breeding pools (Bosch et al., 2009a; EDGEa, 2014). Disease also poses a large threat to this species; Chytridiomycosis has already caused population declines in areas of Central Spain and North-Central Portugal (Bosch et al. 2001; Rosa et al. 2013), and this is believed to be an ongoing threat to the wild population, including populations of subspecies *A. o. pertinax* (Bosch et al., 2009a). There is also potential threat from other diseases such as 'Red-leg-disease' and iridovirus which have already caused mortalities in populations in Carris Lake, Peneda-Gerês National Park, Portugal (Soares et al. 2003; Bosch et al., 2009a; EDGEa, 2014).

Recent research shows novel Ranaviruses closely related to *Ranavirus Common Midwife Toad Virus* (CMTV), causing disease and mass mortality in several different host amphibian species, one of the worst affected being *A. obstetricans*. Population declines have already been noted in Picos de Europa National Park in Northern Spain since 2005 (Price et al., 2014). The distribution of CMTV seems to be limited to those sites where disease and mass mortality have occurred, and likewise mass mortalities have not generally occurred at sites where no CMTV-like has been found, suggesting a strong causal link between the two. It appears that CMTV is also transferable to other species, including the predatory viperine snake *N. Maura* (Price et al., 2014). The introduction of CMTV to several sites and multiple host species across Northern Spain may be the result of human trans-location of amphibian species, or from contaminated equipment or livestock (Price et al., 2014). Evidence shows an increase in the incidence of CMTV-like ranaviruses across Europe affecting several different host species. There is the potential for a response of directional selection against the virus in amphibian species, as exhibited in UK species in response to ranavirosis; however, this

does not always curb the declines induced by virus infection, so even directional selection for resistance or tolerance of viruses may not prevent CMTV having severe impacts on amphibian communities in Northern Spain (Price et al., 2014). In addition to this, as a generalist, the virus appears to be extremely resilient and therefore hard to fight and build immunity to; this makes it an even bigger threat to populations of *Alytes* (Price et al., 2014).

1.4 Diet and feeding behaviour

1.4.1 FEEDING BEHAVIOUR

In the wild *A. muletensis* pick their food from the ground, mainly at night. The striking distance is about 2 cm (Bush, 1993). If the food source is very rich and there is no great risk, they may forage in bright daylight too, e.g. plucking maggots from a goat carcass floating in a pond (G. Garcia, pers. obs.). They do not jump up to catch flying prey items. Individuals eat any arthropods they can catch e.g. spiders, insect larvae and adult insects (G. Garcia, pers. comm.). Larvae feed preferentially by scraping algae growing on the rocks in pools, and on detritus and water plants, but they are also very good filter feeders (Viertel, 1984; Ziegler, 2000).

In the wild *A. obstetricans* feed on beetles, bugs, maggots, caterpillars, woodlice, spiders, worms, slugs, snails, millipedes, harvestmen and other arthropods, with a general variable size of between 2.1 and 28.0 mm (although exceptionally prey items up to 81 mm have been found in their stomachs). Newly metamorphosed specimens preferred prey of about 6 mm, being especially attracted to worm-like prey (Ewert & Burhagen, 1979; Mou, 1985; Lizana et al., 1986; Galán et al., 1990). Stomach content analyses are documented for two populations: 1) in Galicia (Spain) it was found Araneae (25%), beetles (22%), springtails (12%), Diptera larvae (7%), Isopoda (6%), Gastropoda (4%), Diplopoda (3%), larvae of Lepidoptera (3%) and Oligochaeta (2%) (Galán et al., 1990); 2) in Salamanca (Spain) the diet consists of Formicidae (26%), beetles (14%), mites (7%), Araneae (4%), springtails (4%), Lepidoptera (2%) and Diptera (2%) (Lizana et al., 1986). The larvae feed on plants and their debris (WAZA, 2014).

Stomach content analysis of *A. dickhilleni* individuals from Almería (Spain) showed ants (28%), spiders (19%), Coleoptera (11%), Diplopoda (4%) and Hemiptera (4%) (González-Miras et al., 2012). In the wild the larvae feed on plants and even small animals. At high densities the larvae may become cannibalistic (García-Alonso & Escolano, unpublished data in the wild).

In Huelva (Spain), diet of *A. cisternasii* was constituted by 24.2% of Hymenoptera prey (especially ants), Gastropoda (23.5%), Araneae (10.8%), Coleoptera (10.2%), Dermaptera (10.5%) and 8.2% of insect larvae (Mellado, 1976). During winter months (November-February) the diet in southern Portugal consists on insects, spiders and molluscs (Crespo, 1979).

Details on diet and feeding behaviour of *Alytes maurus* in the wild have not been published.

1.5 Reproduction



Fig. 14. (Left) Male *A. cisternasii* with eggs (S. Bogaerts); Fig. 15. (Middle) Male *A. muletensis* with eggs (B. Tapley); Fig. 16. (Right) *A. dickhilleni* with eggs (D. Garcia-Alonso).

Alytes are unique in their reproduction; during mating, the male coils the laid and fertilised string of eggs around his hind limbs (Figs. 14-16); it then carries them on land until embryonic development is completed. The male then seeks out a suitable water body where the larvae are released; this may differ depending on the species. Additional mating may follow and males can carry several clutches at once (Pinya & Perez-Mellado, 2014).

1.5.1 DEVELOPMENTAL STAGES TO SEXUAL MATURITY

In *Alytes*, egg development is terrestrial; it has been suggested that these toads will use this to their advantage, delaying dropping eggs until reliable water bodies are available, as seen in wild *A. cisternasii* (Marquez, 1992).

A. cisternasii larvae are about 13mm upon hatching, and metamorphose when they have reached a maximum length of 70mm (Fig. 17), after 110 to 140 days at a development temperature of 20°C; Newly metamorphosed toadlets have a snout-vent length of 24 mm (Van Der Meijden, 1999a).

A. dickhilleni larval period is typically longer than one year: the larvae over-winter once, meaning that stable, year-round water sources are required for reproduction and growth (Van der Meijden and Cavagnaro, 1999; Bosch et al., 2009b). Larvae metamorphose before winter in low altitude populations (Garcia-Alonso & Escolano, unpublished data) (Fig. 18).

A. muletensis eggs are a cream-yellow colour at first, and translucent enough to see the embryonic development up to 11 days, by which time the embryo will exhibit a response to light (Tonge & Bloxam, 1989). Larvae hatch at the beginning of May, when they are deposited in water (EDGEc, 2014). Total length upon hatching is 18 mm, and the larvae grow up to 76 mm (Fig. 19) in a few weeks (Van der Meijden, 1999c; EDGEc, 2014). Metamorphosis occurs between June and September. Larvae that hatch in October will over-winter before metamorphosing the following summer (EDGEc, 2014; G. Garcia, Pers. comm.). Over-wintered larvae tend to grow larger, and their presence may inhibit the growth of smaller individuals (Moore et al., 2004). There is no brumation period (Van der Meijden, 1999c). When the hind limbs are well developed, the larvae have a total length of up to 50mm (Spence, 2002). Development of larvae is shown to be suppressed by large competitors, high densities and low temperatures (Lea and Halliday, 2002a).



Fig. 17. (Top left) The larva of *A. cisternasii* (Sergé Bogaerts). Fig. 18. (Top right) Larva *A. dickhilleni* (Wouter Beukema). Fig. 19. (Bottom left) Larva of *A. muletensis* (Benjamin Tapley). Fig. 20. (Bottom right) larvae of *A. obstetricans* (Pierre Fidenci).

A. obstetricans larvae hatch upon being deposited, after 3 to 6 weeks of being carried (Engelmann, 1985). Upon hatching, the larvae are about 15mm and attain a maximum length of 80mm (Engelmann, 1985; EDGEa) (Fig. 20). The larvae usually metamorphose the next year, overwintering once (Engelmann, 1985). At sites in the French Pyrenees larvae have been recorded to overwinter for 3 years as indicated by VIE retention (F. Clare, Unpublished data).

Data for the developmental stages of *A. maurus* are currently unavailable.

1.5.2 AGE OF SEXUAL MATURITY

In *A. cisternasii* sexual maturity is reached when the frog is at least 2 years old (Noellert & Noellert, 1992).

A. muletensis males on average reach sexual maturity at 12 months, females at 24 months (Pinya & Perez-Mellado, 2013).

In captivity *A. dickhilleni* reach sexual maturity at 24 months post metamorphosis (Garcia-Alonso & Robledo, Unpublished data.)

Data for age at sexual maturity for *A. maurus* and *A. obstetricans* are currently unavailable.

SEASONALITY OF CYCLING

A. cisternasii females can produce up to four clutches per breeding season (Van der Meijden, 1999a), which occurs between September and March throughout its range (Noellert & Noellert, 1992). Reproduction of *A. cisternasii* populations in Southern Spain was concentrated into discrete peaks

(Lopez-Jurado et al., 1979), one of calling and amplexus (October), and the other of egg release (November) (Marquez, 1992); however a population in Central Spain was found to have an explosive (short) breeding season (Rodriguez Jimenez, 1984). Marquez (1992) estimates that low winter temperatures may end the breeding season, which is predominantly in autumn and begins with the first rains. It is thought that a lack in spring breeding in wild populations of *A. cisternasii* may be correlated with a long larval development which would be interrupted when streams dried up in the summer (Marquez, 1992).

In *A. dickhilleni* reproductive behaviour is highly variable. In some populations males start calling in December (Antunez et al., 1982; Garcia-Alonso & Escolano, unpublished data). In the wild *A. dickhilleni* start calling in March (Martinez-Solano et al., 2003). In some populations eggs are laid from May to September (Martinez-Solano et al., 2003). And eggs are laid from February to September In Malaga eggs are laid from February through to September (Garcia-Alonso & Escolano, unpublished data). Some larvae metamorphose between August and October, others overwinter and metamorphose the following year; it does not appear that date of hatching is correlated with the larvae overwintering (Martinez-Solano et al., 2003).

In *A. muletensis* there is an extended breeding season from May to September in the wild, and can be year-round in captivity, during which time males may fertilise and carry eggs from several females (Lea et al., 2002b; Pinya & Perez-Mellado, 2014). Egg carrying males are found mostly in May and June (Noellert & Noellert 1992).

The mating season of *A. obstetricans* varies throughout the range. In Westfalen, Germany, egg carrying males can be found between the end of March, and the beginning of August; in Spain, around the city of La Coruña, egg carrying males have been observed from mid-February through to August; Mountain populations of male *A. obstetricans* can carry eggs well into August (Van der Meijden, 1999b). In some German and Spanish populations, there are several peaks of breeding activity over a breeding season which may last several months; high elevation populations are restricted in their activity to short seasons due to extreme environmental conditions, and it has been found that snowmelt in late spring triggers the start of the breeding season in montane populations (Angelier and Angelier, 1964; Marquez, 1992).

There is no information on the seasonality of cycling for *A. maurus*.

1.5.3 GESTATION PERIOD/ INCUBATION

Wild *A. cisternasii* males are known to carry egg clutches for up to 30 days in conditions of optimal temperature and moisture, although they may carry them for longer in sub-optimal conditions; in one study males were found to carry eggs for up to 20 days under suitable conditions and up to 32 days when rains were delayed (Marquez, 1992).

A. muletensis eggs hatch after 11 to 36 days, hatch time varying with temperature although the 11 days documented in this publication are likely to be an erroneous record (mean 21.75 days; Tonge & Bloxam, 1989).

A. obstetricans hatch 20 days after fertilisation when kept at a constant temperature of 20°C (Cambar and Martin, 1959) and it is estimated that this would equate to a period of 26 to 32 days in the wild, where soil temperatures would likely be lower in the microhabitat where males incubate the eggs (Marquez, 1992).

In captivity *A. dickhilleni* carry eggs for an average of 22 days (A. Escolano, Pers. comm.).

Information on incubation period is unavailable for *A. maurus*.

1.5.4 CLUTCH SIZE

Species	Average	Range	Reference
<i>A. cisternasii</i>	73	42-119	Crespo, 1979
	73	48-116	Malkmus, 1983
<i>A. dickhilleni</i>	74	18-149	Gonzalez-Miras et al., 2012; Garcia-Alonso & Escolano, unpublished data.
<i>A. maurus</i>	-	60-70	Donaire-Barroso & Bogaerts, 2003
<i>A. muletensis (wild)</i>	12.04	4-34	Pinya & Perez-Mellado, 2014
	9.78	7-12	Alcover et al., 1984
<i>A. muletensis (captive)</i>	-	10-24	Martinez-Rica et al., 1984
	11.43	9-15	Tonge & Bloxam, 1989
	11.36	4-27	Bush, 1996
<i>A. obstetricans</i>	63	35-95	Crespo, 1979
	51	24-142	Buchholz 1989
	38.5	5-120	Galan et al., 1990
	77	32-171	Reading & Clarke, 1988

Table 4 Clutch size in *A. cisternasii*, *A. dickhilleni*, *A. maurus*, *A. muletensis* and *A. obstetricans*. Adapted from Pinya & Perez-Mellado (2014).

Clutch size in *Alytes*, as shown in Table 4, is variable, both inter- and intra-specifically (Pinya and Perez-Mellado, 2014). It may refer to the number of eggs laid by a female, or to the number of eggs carried by a male, which can include clutches from several different females.

A. cisternasii males can carry up to four clutches with a total of 180 eggs or more (Noellert & Noellert, 1992).

A. maurus produce 3-4 clutches per breeding season (Donaire-Barroso et al., 2009).

Maximum clutch size in captive *A. muletensis* is 34 (Roman & Serra, 1997).

Male *A. obstetricans* can carry up to three clutches with a total of 150 eggs or more (Schleich et al., 1996). Marques (1992) found that in the wild, the number eggs contained in each clutch released by a male decreased over the June to August mating period; this may have been due to a decrease in the number of matings per male rather than a decrease in number of eggs laid per female. Marquez (1992) also found that wild male *A. obstetricans* may carry a second clutch of eggs after hatching out the first.

1.5.5 HATCHING DETAILS AND SEASONS

Alytes males may hatch out more than one clutch per breeding season in the wild, although according to Marquez (1992), environmental and physical constraints may prevent this being

possible: males may be physically unable to carry many egg clutches at once due to their size/ the size of a single clutch; also males may delay dropping their eggs if conditions are not suitable for egg hatching; finally males must release all the eggs in their load at once, all of which must be at a stage where they are ready to hatch. These factors therefore limit the opportunity for males to obtain multiple clutches without affecting hatching success (Marquez, 1992).

Marquez (1992) estimates that under the environmental conditions *A. cisternasii* experience in the wild, it is unlikely a male will hatch out more than one batch per mating season owing to the synchrony in matings (i.e. all matings occur before clutch release).

A. dickhilleni larvae hatch out from February May to September (Martinez-Solano et al., 2003; García-Alonso & Escolano, unpublished data). In some population there are two hatching periods, the first one in February-March and the other in September (García-Alonso & Escolano, unpublished data).

A. maurus eggs are carried around on land by the male until they are ready to hatch, at which point they are released into a suitable water body (Donaire-Barroso et al., 2008).

A. muletensis eggs hatch after 11 to 36 days, varying with temperature (mean 21.75 days, Tonge & Bloxam, 1989). The first larvae hatch in the beginning of May, with metamorphosis occurring mostly in June (Noellert & Noellert, 1992).

A. obstetricans larvae hatch after 3 to 6 weeks. The males seek out small water bodies to discard the egg strings with the hatching larvae (Engelmann, 1985). Marquez (1992) found that in the wild the proportion of eggs hatching per clutch increased over the June to August mating season; this may be due to higher temperatures towards the end of the season producing faster egg development and therefore less exposure time to fungal infection.

1.6 Behaviour

1.6.1 ACTIVITY

Captive male *A. cisternasii* start calling in October, as is expected in their natural habitat, when the first autumn rains start (S. Bogaerts, pers. comm., 2014). During the breeding season calling occurs for a few hours every evening. Males produce advertisement calls and females reply using lower intensity calls (Van der Meijden, 1999a). Marquez (1992) found that daily activity in the wild was not affected by maximum or minimum temperature. They are primarily nocturnal (Marquez, 1992).

The call of *A. maurus* is a short pure tone, similar to that of *A. obstetricans*, lower in frequency than *A. muletensis*, and shorter than *A. cisternasii* and *A. dickhilleni* (Marquez et al., 2011).

A. muletensis males call during the mating season, a short, high-pitched, metallic call, repeated every one to three seconds. They get their local name 'Ferreret' from their vocalisations; Ferreret means little metal worker in Balearic Catalan and Spanish, and their call apparently sounds like metal being hit with a hammer. Calling occurs between late February and late August. All parts of the courtship

are terrestrial: males call from rock crevices and females answer vocally, before approaching them (Bush, 1993). *A. muletensis* is primarily nocturnal, with young and adults spending the majority of the day hidden in limestone rock crevices (Bush, 1993). Larvae may be exposed to varying levels of light in their pools, and have been observed being active during the day, feeding and swimming; this activity increases in the afternoon (Spence, 2002). They are a thigmotaxic species, and in captivity groups of up to 13 adults or 70 newly metamorphosed juveniles may congregate under a single piece of refugia (Bush, 1993). Adults may move between pools within the same torrent, but are probably unable to cross open territory when seeking new breeding sites.

Tadpoles of *A. muletensis* display a clear diel pattern of activity and microhabitat selection in torrent pools in Mallorca. Individuals spent much of the day foraging (not aggregated) in warm shallow areas, with the peak of activity at 17:00-19:00 hr. In mid-afternoon, however, when much of the water surface is in direct sunlight, tadpoles move away from the shallows and form aggregations under rock overhangs at the sides of the pool. Both activity and tadpole density in the shallows drop dramatically after dark, when there is a movement of the population back into deeper water or hiding places (Schley et al., 1998).

Males of *A. obstetricans* start calling from their shelters before sunset, and males and females return to seek shelter before dawn (Marquez, 1992). The call is a high-pitched, explosive, musical "poo...poo...poo..." about one call every 1-3 seconds (Engelmann 1985). Marquez (1992) found that daily activity in the wild was not affected by maximum or minimum temperature; however montane populations of the toads become more active with the melting of snow at the beginning of the breeding season in late spring. Larvae in mountain areas often overwinter as larvae and can remain dormant or be active under a thick layer of ice (Campeny & Casinos, 1989).

A. dickhilleni activity is mainly crepuscular and nocturnal (Marquez and Bosch, 1996). In low elevation areas adults can be active all year while in high mountains its annual activity is restricted to the warmer months between March and October (González-Miras et al., 2012). Specimens have been observed basking at midday in the wild (García-Alonso & Escolano, unpublished data).

1.6.2 LOCOMOTION

Alytes cisternasii and *A. obstetricans* are fossorial anurans that primarily use their forelimbs in burrowing. The manoeuvrings are quite dexterous and the forelimbs are used alternatively or sometimes synchronously. The fingers are scraped into the substrate, and occasionally the head is pushed into the substrate or acts in a scoop-like manner. Both species are known to construct a system of tunnels and cavities underground, and they do push-ups packing the substrate against the top of the tunnel with their head (Brown & Crespo, 2000). The two species are quite similar in motor patterns of forward burrowing. However, *A. cisternasii* makes minimal use of its hind limbs (only for bracing), whereas *A. obstetricans* actively uses its hind limbs to kick soil posteriorly to the surface. Also, *A. cisternasii* is a rapid, efficient forward burrower that is highly fossorial, whereas *A. obstetricans* is a reluctant forward burrower that lingers on the surface and prefers pre-existing holes (Brown & Crespo, 2000).

A. cisternasii do not climb well (S. Bogearts, pers. comm.).

A. muletensis climb very well and cover downward distances by dropping rather than climbing (Moore et al., 2004).

A. dickhilleni climb very well. They have been observed climbing on smooth concrete walls and natural rock walls (Garcia-Alonso & Escolano, pers. comm.). It is not known if the species can construct tunnels underground (Garcia-Alonso & Escolano, pers. comm.). They appear to use only their hind limbs to kick and push soil first to create indentations in the substrate in which they sit. Once they have removed some substrate they then check the size of the cavity with their head and forelimbs and may dig further with the forelimbs until the cavity is the correct size. They repeat this routine many times and finally they obtain a little hole with the size of the toad for to be there (Escolano & Garcia-Alonso, unpublished data).

There is currently no information available on the locomotion of *A. maurus*.

1.6.3 PREDATION

Predators of *A. cisternasii* larvae include introduced fish species and the Louisiana crayfish *P. clarkii* (Beja et al., 2009). At sites of egg release for *A. cisternasii* in South-Central Spain, dragonfly and *Dytiscus* larvae, water snakes (*N. maura*) and water turtles (*Mauremys leprosa*) were common and potential predators of larvae (Marquez, 1992).

A. maurus larvae are preyed on in their native habitat by introduced fish species *G. holbrooki* (Donaire-Barosso et al., 2009).

The only known native predators of *A. muletensis* are owls (Bush, 1993), although Corvids (Magpies and crows) have the potential to take toads opportunistically. Wild larvae have shown adaptation to introduced predatory viperine snakes (*N. maura*) by developing faster and developing longer tails with narrower fins and deeper muscles; this presumably improves their chances of escaping (Moore et al., 2004). Research shows they may also avoid predation through reducing activity in response to chemical cues from *N. maura* (Schley & Griffiths, 1997). It is thought that the *A. muletensis* have recently evolved the ability to avoid predation from *N. maura*, as this ability appears to be genetically determined rather than learned; post-metamorphic individuals can detect and actively avoid those sites which contain predatory snakes (Schley & Griffiths, 1997). In their wild habitat chemical cues are likely a more reliable indication of predatory presence than sight as the toads tend to inhabit rock crevices and other dark areas (Schley & Griffiths, 1997).

Predators of *A. obstetricans* include *L. gibbosus* and other introduced predatory fish species, including salmonids (Bosch et al., 2009a). Larvae at certain sites in the Spanish Pyrenean Mountains were only known to be preyed by water beetles (*Dytiscidae*) and birds visiting the pond to feed on juvenile *Rana temporaria* (Marquez 1992). In the French Pyrenees dragon fly larvae have been

observed to feed on both larval and newly metamorphosed *A. obstetricans* (F. Clare, unpublished data).

1.6.4 SOCIAL BEHAVIOUR

A. muletensis individuals use phonotaxis to communicate, locate a mate or find their way to a breeding sight; it is thought that phonotaxis does not always necessarily show a motive for mating (Lea et al., 2002b).

Alytes cisternasii and *A. obstetricans* form aggregations (2-5 individuals) underground suggesting social interactions (Brown & Crespo, 2000).

Guyétant (1973) showed that larval development and metamorphosis of *A. obstetricans* are different according to whether the individuals are living in groups or in isolation. The tadpoles living in groups are far bigger than the isolated ones and their metamorphosis occurs earlier.

There is no information on the social behaviour of *A. dickhilleni* and *A. maurus*.

1.6.5 SEXUAL BEHAVIOUR

In *Alytes* species, the male will attract females with calls, and the female will approach the male, after which a complex amplexus will occur on land (Marquez et al., 2011). Amplexus in *Alytes* is inguinal (Wells, 2007).

A. cisternasii females respond to male advertisement calls by seeking out the male, who then grasps the female round the lumbar region, and the two individuals then rock from side to side and forward and backward (Fig. 21). After some time the male tightens his grip suddenly, causing the female to release the eggs into the trough between her hind legs, which are then fertilised by the male. The male then distends the egg mass and winds them around his ankles (Noellert & Noellert, 1992; Van Der Meijden, 1999a).



Fig. 21. (left) Amplexant pair of *A. cisternasii* (S. Bogaerts); Fig. 22 (right) Amplexant pair of *A. o. boscai* (M. Vences & F. Glaw, 1989)

A. muletensis individuals sit in amplexus, while the male 'pedals' his legs against the female's cloaca, and the female 'pivots', to which the male responds by nodding his head; this amplexus can be terminated by either individual, and it has been observed that females will sometimes interrupt the courtship of other individuals (Bush, 1993).

In *A. obstetricans* the courtship and mating process is very similar to *A. cisternasii*, in that the female will respond to the male's call, and once located, the male grasps the female around the lumbar region and stimulates her cloacal region with his toes (as seen in the pair of *A. o. boscai* in Fig. 22). The male constricts his grip on the female and she releases an egg mass, which the male fertilises with liquid sperm. The eggs are then wrapped around the ankles of the male, and carried until they are ready to hatch (Van der Meijden, 1999b).

There is currently no information on the sexual behaviour of *A. dickhilleni* or *A. maurus*.

SECTION 2: MANAGEMENT IN ZOOS AND AQUARIUMS

2.1 Enclosure

Larvae, juveniles and adults can be kept in glass or plastic aquaria. Some *Alytes* species are agile and likely to climb tank walls in captivity, a securely fitting lid is strongly advised (C. Michaels, pers. comm.). Caution is taken when removing lids for service to ensure the toads do not get damaged or escape.

2.1.1 Basic maintenance

The toads must be checked every day in order to monitor their health and the general status of their enclosure; any old food and faeces must be taken out each time the enclosure is serviced. Substrate must be cleaned on a regular basis; frequency of substrate changes will be dependent on the stocking density, size and activity levels of the toads.

Animal husbandry should aim to improve the long-term fitness and viability of individuals and populations (Gascon et al., 2007). Problems in captive husbandry and breeding can usually be traced back to gaps in the fundamental understanding of species biology, ecology, behaviour, phenology and the physical parameters of preferred habitats (Michaels et al., 2014). If conservation breeding and captive breeding and conservation research programmes that utilise captive bred and reared amphibians are going to yield meaningful results captive management needs to be informed by field data.

2.1.2 Substrate

Larvae do not need to be provided with substrate, but sand and gravel may be provided if desired. Juveniles and adults require substrate, which can be gravel (e.g. granite or limestone). For *A. cisternasii*, a substrate of mixed sand and loam of about 5cm has been used successfully, as these species are known to dig; Juveniles can be kept in tanks with small/fine gravel or soil substrate of 1 cm to allow them to be checked with ease (S. Bogaerts, pers. comm. 2014).

If specimens are to undergo a cooling period the substrate will need to accommodate digging activities of the toads. A mix of sand and loam with additional cover objects such as bark is suitable.

It is important to provide a humidity gradient within the enclosure; if animals are permanently wet they can develop skin conditions (G. Garcia, pers. comm.).

2.1.3 Furnishings and maintenance

A number of furnishings can be used to mimic the toad's natural environment, although they need not be too elaborate (Tonge & Bloxam, 1989). Basic shelters such as earthenware roof tiles and air bricks (see e.g. ZSL enclosures in Fig. 23), should be placed in the enclosure, and in captivity these are used regularly by *A. dickhilleni*, *A. muletensis* and *A. obstetricans*. PVC pipes with diameter up to 50 mm can also be used to provide refugia for the toads. Provision of refugia is important, as *Alytes* species are primarily nocturnal and therefore require shelter during the day. They are also thigmotactic, meaning they tend to gather under refugia (often in groups and maintain close contact with one another, possibly to retain skin moisture levels) which the dorsal surface comes into

contact with. *A. muletensis* have been provided with rocks, logs and branches at Durrell, although these did not appear to affect breeding success (Tonge & Bloxam, 1989). It is important to provide a wide range of refugia both in size and location so that toads can choose from a range of humidity levels throughout their enclosure.



Fig. 23 Enclosures for *A. muletensis* and *obstetricans* adults at ZSL London Zoo (B. Tapley)

Alytes larvae should be provided with shelters (e.g. unglazed terracotta, Cored/ hollow/ perforated bricks); this allows individuals to hide and also provides a surface for algae growth which the larvae may feed on. Plants may also be added to mimic the natural habitat of the larvae (Fig. 24). It is important that porous items in the enclosure are not cleaned with chemical disinfectants and re-used as these may leach into the aquatic environment.



Fig. 24 Enclosure for *A. muletensis* larvae at ZSL London Zoo (Benjamin Tapley)



Fig. 25. Dedicated breeding facility for *A. dickhilleni* at Bioparc Fuengirola (D. Garcia-Alonso)

An important consideration when selecting enclosure furnishings is the ease at which all animals within the enclosure can be checked. Keepers should be able to move furnishings with ease and all furnishings must be firmly placed to avoid crushing the frogs.

As the life history of *Alytes* has both terrestrial and aquatic elements, they should be provided with both a terrestrial area and an appropriately sized water body. The adult life stage is predominantly terrestrial, therefore the majority of the enclosure should be terrestrial. Constant water sources are also important particularly as this is where the males will deposit the eggs when they are ready to hatch. *Alytes* in captivity are also observed spending prolonged amounts of time in water. A land-water ratio of 2:1 was used successfully at Durrell (Tonge & Bloxam, 1989), and at ZSL London Zoo the substrate is kept moist but dry areas under refugia are always provided for the toads. Plants may be added to water to absorb nitrates (Odum & Zippel, 2008), although caution is advised in the use of water plants, which should not be taken from areas known to be inhabited by other amphibians due to the risk of introducing harmful pathogens.

2.1.4 Environment

Alytes should be kept in rooms with strict temperature controls; temperature regimes should be as close as possible to those experienced by the toads in their natural environment (a range of 7°C and 25°C was deemed appropriate by Tonge & Bloxam (1989) for *A. muletensis*). Individuals were kept and bred successfully in an outside enclosure at Durrell (Tonge and Bloxam 1989), although this is not recommended due to risk of individual escape and disease transmission to and from native species. It is advised that conditions are kept as close to those in the toads' natural range as is possible, as this will facilitate a natural breeding cycle. This may involve providing a misting system, such as that shown in Fig. 25-26.

Water temperature, pH, dissolved oxygen and nitrogenous waste compounds are important water parameters to monitor, particularly when working with *Alytes* larvae, as they are known to affect larval development (McDiarmid & Altig 1999; Lea et al., 2002a).



Fig. 26. Housing for *A. muletensis* at Durrell (G. Garcia)

2.1.5 SEASONALITY

In order to keep the *Alytes* in conditions as close to those they would experience in the wild state as possible, climate controls should be set to mimic seasonality, through temperature fluctuations and rainfall, which can be achieved through the use of sprinklers.

Sprinklers were used to mimic rainfall for *A. muletensis* at Durrell (Fig. 20), and a more heavy-spray regime was implemented in the autumn and winter, mimicking conditions in Mallorca (Tonge & Bloxam, 1989). *A. cisternasii* have been noted to start calling with the first autumn rains in captivity (S. Bogaerts, pers. comm.), which correlates with behaviour seen in wild populations, in which the first peaks in breeding activity coincide with the first Autumn rains (Marquez, 1992).

2.1.6 UV, LIGHT AND HEAT

UV, light and heat are all important components of amphibian husbandry and these must be provided as a gradient in the enclosures (Tapley et al., 2015). Being ectothermic, *Alytes* require different microclimates in the captive environment in order to self-regulate their body condition.

In the wild *Alytes* are primarily nocturnal, spending the daylight hours hidden in rock crevices and have not yet been observed basking; it is thought they may be exposed to certain levels of reflected UVB radiation. Clinical symptoms of metabolic bone disease (MBD) have been exhibited in specimens which have not had any exposure to UVB radiation; although in these cases sub optimal captive diets cannot be ruled out as a contributing factor. MBD can be detected through radiography and manifests in the form of bone curvature and poor mineralisation. At ZSL the *Alytes* are provided with Arcadia T5 lamps with 12% UVB (D3+ 12%UV-B 121 Reptile Lamp, Arcadia Products plc, Redhill, UK), which gives a gradient of UVB radiation and light, and is controlled throughout the day to correspond with the fluctuations in temperature; the toads are exposed to a UV Index (UVI) of 1.0 as this genus is considered shade-dwelling.

In Nature, larvae are exposed to different levels of UVB radiation according to the position of the pool in which the larvae are deposited, water depth, turbidity of the water and availability of refugia.

The UVB and vitamin D₃ requirements of amphibians are still largely unknown. Vitamin D₃ can be obtained by its ingestion in food but in most vertebrates is produced endogenously by exposure to natural sunlight: exposure of the skin to UVB radiation from sunlight, specifically of UVB wavelengths between 290 and 315 nm. Low vitamin D₃ levels result in insufficient calcitriol (the active hormone enabling calcium absorption from the gut) being produced and, as a consequence, serum calcium levels fall. The resulting hypocalcaemia stimulates production of parathyroid hormone, which in turn promotes calcium resorption from the bones and results in generalised skeletal decalcification. Skeletal decalcification is reversible although any deformities may be permanent.

Providing suitable gradients of UVB in captivity is vital, and will facilitate self-regulation of UVB exposure by the individuals. However, an over-exposure can be detrimental (Antwis & Browne, 2009). The exact UVB requirements of *Alytes* are currently unknown. At ZSL a UVI of 1.0 is provided to *Alytes*, based on the fundamentally nocturnal and shade-dwelling habits of this genus.

It is important to take regular UVI readings using a UVI Meter in order to calculate the level of radiation the toads are being exposed to, because the level of UVB emitted by UVB emitting lamps will deteriorate over time. This can be achieved using e.g. a Solarmeter 6.5 UV Index Meter (Solartech Inc., Harison township, MI, USA). In addition to this, routine health screening of individuals through radiography is advised in order to assess skeletal health.

2.1.7 Dimensions and stocking density

For *Alytes* larvae a rearing density of 1 individual per litre is deemed reasonable due to the relatively large size of *Alytes* larvae. Low growth rates have been documented in *A. muletensis* and *A. obstetricans* kept in small groups (G. Garcia and G.M. Rosa pers. comm.). All closed systems will require regular water changes (Browne & Zippel, 2007), although the regularity will depend on factors such as the water temperature, activity, and the type and amount of food offered, as these factors will determine the amount of nitrogenous waste that will be produced by larvae. The development of captive and wild larvae is affected by larval density, light levels, food provision, temperature, pH and dissolved oxygen levels of the water; it is therefore important to provide the right stocking density in relation to these other factors in order to promote healthy growth of individuals (McDiarmid & Altig, 1999).

At ZSL London zoo no more than 15 post metamorphic *Alytes* are maintained in enclosures measuring 51 x 51 x 48 cm (of which 1/4 of the floor area is water) with ample refugia.

2.2 Feeding

Only live food is accepted by post-metamorphic *Alytes*. Where possible it is recommended that the toads are fed in the evening or at night, to coincide with the nocturnal activity of these toads. If keeping toads under seasonal conditions, feeding may vary; toads may be fed 3 times a week in the summer and once per week in the winter as a general rule. At Durrell *A. muletensis* individuals were fed five to seven times a week in summer, two to three times a week in autumn and not at all during the winter i.e. November to February as toads became inactive due to the cool temperatures (Tonge & Bloxam, 1989). If toads are being bred for potential release in the wild, live-food should be bred in the institution the frogs are being held in, rather than buying from commercial breeders who often stock both reptiles and amphibians, to avoid disease transmission (Tonge & Bloxam, 1989; Serge Bogaerts, pers. comm. 2014). It is recommended that each individual is given 3 to 5 food items each at every feed. This may need adapting if individuals are seen to be below a healthy weight; in extreme cases very skinny individuals (i.e. individuals in which the urostyle is protruding) may need removing and keeping on their own so their diet can be monitored more easily. Females in the breeding season may also need to be provided with additional food.

2.2.1 Basic Diet

Post metamorphic toads can be offered a variety of small invertebrates; this may include flightless fruit-flies (*Drosophila hydei*), pinhead and house crickets (*Acheta domesticus*), wax-moths (*Achroia grisella*), aphids (Aphidoidea), bean weevils (*Sitona lineatus*) and spiders (Araneae). *A. cisternasii* have been reared successfully on *Alphitobius laevigatus* larvae (S. Bogaerts, pers. comm. 2014). As a general rule on the size of the food given to amphibians, the invertebrates should not be larger (i.e. longer) than the distance between the eyes of the individuals in question.

Knowledge of rearing methodology for tadpoles of many species is entirely lacking (see review in Pryor, 2014). The standard diet for captive *Alytes* larvae as applied at Durrell and ZSL London Zoo is as follows:

- 10 grams fish flakes
- 8 grams trout pellets
- 8 parts grass pellets
- 3 grams cuttlefish bone
- 1-2 grams Tubifex
- 1-2 grams river shrimps
- 2-3 grams Spirulina algae

For which 'parts' are measured in weight. These ingredients should be combined and reduced to a fine powder which can be sprinkled onto the surface of the water. The larvae may also feed on algae growing on tank surfaces.

For larval diets care should be taken to ensure that the correct brand of fish flake is selected. For Bufonids a diet of consisting of 46% protein is the best in terms of optimal growth (Martins et al. 2013).

2.2.2 Special dietary requirements

Dietary supplementation (e.g. nutrobal) is required to counteract the nutritional deficits in the diet of captive amphibians. Invertebrate species that are commonly cultured are cultured because they breed readily in captivity, not because they are nutritionally sound prey items. Prey items should be dusted with dietary supplements immediately before being fed out to amphibians. Dietary supplements often oxidise if they are exposed to the air and humidity, and they are also photosensitive. Supplements should be kept in a dark fridge in an airtight container. All food items should be supplemented.

2.2.3 Method of feeding

Food should be scattered in the enclosure as late in the day as possible, as *A. cisternasii*, *A. muletensis* and *A. obstetricans* are known to be nocturnal and most feeding activity will occur at night; this means that feeding at night is particularly important to ensure maximum uptake of dietary supplements which may wear off the longer food is left untouched. Uneaten prey items should be removed the following day as supplements may have rubbed off and the gastrointestinal tract of the prey item will no longer contain food and will be of limited nutritional value.

If individuals appear skinny they should be separated from conspecifics for feeding.

Food for larvae can be sprinkled on the surface of the water; feeding times are not as important for larvae as they are known to be active during the day.

2.2.4 Water

WATER REQUIREMENTS

Water quality is an important consideration in keeping any amphibians, as all rely on some form of moisture, be it in the terrestrial or aquatic form (Odum & Zippel, 2008). Monitoring water quality is vital to successfully rearing healthy captive amphibians (Odum & Zippel, 2008); fluctuating water parameters create stress for the individuals, therefore it is better to maintain constant conditions, even if these are slightly sub-optimal.

TESTING AND TREATMENT

It is important to know certain parameters of the water source; sources can be tested for dissolved substances, pH and hardness, and treated if necessary to provide the appropriate conditions. Unfortunately the optimal water parameters are unknown for most amphibian species, although general guidelines are provided by Odum & Zippel (2008) (Table 5). Most institutions use municipal water sources which are generally treated with chlorine, or chloramines; levels of these chemicals should be tested for and treated accordingly (Table 5). Other water sources should be treated with caution; avoid using water which has previously been in contact with other amphibians as this may risk disease transmission, or may have been contaminated by dissolved substances (Odum & Zippel, 2008). Reverse Osmosis (RO) systems will produce water that is too pure for amphibians and using pure RO may cause osmoregulatory imbalances. RO water can be made suitable either by adding salts or a known quantity of tap water.

Water quality parameter	Recommended levels	Control methods	References
Water hardness (dissolved Ca and Mg salts)	For soft water amphibians: <math><75\text{mg litre}^{-1}</math> (ppm) of CaCO_3 . For hard water amphibians: >100mg litre ⁻¹ of CaCO_3 .	Soft water: Harden using Ca and Mg salts (only recommended reconstituting RO (reverse osmosis), DI (de-ionized) or distilled water). Hard water: Soften using RO, DI or distilled water.	Whitaker, 2001
Dissolved oxygen as O₂	>80% saturation	Aeration	Gulidov, 1969 Brungs, 1971 Carlson & Siefert, 1974 Siefert & Spoor, 1974 Siefert et al., 1975 Odum et al., 1984 Whitaker, 2001
Gas supersaturation	Gases maintained at equilibrium with the atmosphere.	Aerate water until equilibrium with atmosphere is achieved.	
Ammonia/ Ammonium-NH₃/NH₄⁺	<0.2mg litre ⁻¹ , N as unionized ammonia	Biological filtration, chemical filtration (with appropriate medium), water changes.	Tabata, 1962 Herbert & Shurben, 1965 Ball, 1967 Jofre & Karasov, 1999 Rouse et al., 1999 Whitaker, 2001
Nitrites NO₂⁻	<1.0mg litre ⁻¹ , ideally 0	Biological filtration, chemical filtration (with appropriate medium), water changes.	Klingler, 1957 Russo et al., 1974 Westin, 1974 Marco et al., 1999 Whitaker, 2001
Nitrates NO₃⁻	<50.0mg litre ⁻¹	Removal: photosynthetic action of green plants or water changes.	Westin, 1974 Whitaker, 2001
pH	Generally near neutral, although it is species-dependant. Should avoid pH <6 and >8.	Change water source or add appropriate buffer solution.	Cummins, 1989 Warner et al., 1991 Whitaker, 2001
Chlorine Cl₂	0	Aerate for 24 hours, or add chemical dechlorinator such as sodium thiosulphate.	Arthur & Eaton, 1971 Culley, 1992 R.A. Odum, pers. obs.
Chloramines (ClNH₂, Cl₂NH, ClN₃)	<0.01mg litre ⁻¹ as Cl	Use chemical treatment specific for chloramines such as Prime® (Seachem Laboratories, Inc., Madison, GA 30650, USA). Filters for this purpose are available.	Environmental Protection Agency (EPA), 1976 Odum & Zippel, 2008
Copper (Cu)	<0.05mg litre	Carbon filtering and carbonate precipitation (do not use copper piping).	Pritchard-Landé & Guttman, 1973
Posphates (PO₄³⁻)	Toxicity species-specific; EPA recommends limit of 10mg litre ⁻¹ ; 1mg litre ⁻¹ is effective for preventing pipe corrosion.	Lower levels of phosphates using phosphate sponges and filters.	

Table 5. Water quality parameters: guidelines for keeping amphibians. Adapted from Odum & Zippel (2008).

Water parameters should be tested frequently in newly established systems, and can become less frequent as the system develops (Odum & Zippel, 2008). Testing should be carried out on a regular basis in order to monitor the effectiveness of biological filtration.

FILTRATION

The most common system used for keeping aquatic animals is a semi-closed system, in which a combination of filtration and water changes are employed in order to maintain water quality (Odum & Zippel, 2008). Water changes should be small and regular (no more than 20% of the original volume of the water body in question). Rapid changes in water parameters can be highly stressful on the physiology of aquatic organisms. The necessary frequency of water changes will depend on the stocking density of larvae, the temperature of the water and the amount and type of food given at each feed, as these have an effect on the level of activity within the tank, which in turn affects the amount and character of waste material produced.

Suspended particles can be removed from the water column through mechanical filtration, dissolved harmful substances (such as chlorine) through chemical filtration, and nitrogenous waste material produced by aquatic organisms and the breakdown of organic material can be processed by bacteria living on filter media, through the process of biological filtration. This can be achieved through using separate filters, or single filters with combined functions (Odum & Zippel, 2008). Filters should not create a strong flow within the system, as *Alytes* larvae do not occur in fast flowing torrents and therefore will not tolerate strong currents.

The size and type of filter used will depend on the size of the enclosure, volume of water and density of individuals being catered for. At ZSL London Zoo external EHEIM filters (Plochingen Str. 5473779, Deizisau, Germany) are used for large tanks and inline airstream filters for smaller systems. If larvae are being kept at relatively high densities, large external filters may be necessary to remove waste material; for those kept at lower densities (under 20 individuals per litre, Browne & Zippel, 2007), box filters will be adequate.

Filters require regular maintenance in order to ensure they continue to function effectively. The frequency with which filters need servicing depends on the stocking density, the amount of food given per individual per feed, as well as the temperature of the water, as this will affect how much waste material is produced.

Mechanical filters remove physical particulates from the system, and these then build up in the filter if they are not cleaned out straight away. Build up should be avoided as it will reduce the capacity of the filter for dealing with further suspended particles, and any organic matter within them may begin to break down and leach harmful substances back into the system (Odum & Zippel, 2008).

Chemical filters are designed to remove dissolved substances such as organic compounds from the water as it passes through; activated carbon has been identified as an ideal material for amphibian systems and is commonly used as a chemical filtration medium (Odum & Zippel, 2008). It is vital that such filter systems are used in combination with a mechanical filter, as build-up of particulate matter will severely compromise their effectiveness. Chemical filter media will need to be routinely replaced.

Biological filtration systems consist of a community of living organisms which act to break down toxic metabolic waste products in the water to less toxic substances; toxic ammonia is broken down to less toxic nitrite which then in turn is converted to less toxic nitrate (which in itself is still less toxic to aquatic organisms). These filters are therefore the most important component in maintaining the

health of the aquatic system and the amphibians themselves (Odum & Zippel, 2008). Owing to the living component of this filtration system a constant supply and flow of water is required in order to ensure nitrogenous waste is absorbed. An advantage of this is that a slow steady flow is recommended for both the biological filter and the *Alytes* toads and larvae. Biological filters take time to mature and can be seeded with filter media from established systems, this may risk introducing disease causing agents into new set ups. Alternatively filters can be matured using an ammonium chloride dosing regime.

Note that filters are less efficient at processing nitrogenous waste in soft water and at low temperatures.

2.3 Social structure

Not much is known about *Alytes* in terms of territoriality and other social aspects as they are a fairly reclusive genus, although they are known to be thigmotaxic, spending the day hidden in small groups in crevices, males and females mixed (Lea et al., 2002b). In captivity, juveniles are generally raised in separate tanks to the adults for ease of management and feeding (E. Wells, pers. obs.; S. Bogaerts, pers. comm. 2014).



Fig. 27. Larval rearing for *Alytes* at Barcelona zoo demonstrating the use of a floating fish hatchery to separate different larval age classes (G. Garcia).

Larval development is known to be affected by density and cohort structure (Lea et al., 2002a), so caution should be taken to ensure all individuals receive the appropriate amount of food. Different age classes may be reared in the same enclosure with the use of floating fish hatcheries (Fig. 27).

2.3.1 Changing social structure

It is important to know the sex of individual toads in order to establish an appropriate group structure within the enclosure if breeding is desired. Identifying male *Alytes* is easier after breeding,

as they will be carrying the egg strings on their hind legs. Females may be identifiable. If females are gravid, the eggs can be seen through the ventral surface.

If animals are kept in large groups males may drop clutches they are carrying in order to acquire new clutches. Having a male bias sex ratio may lead to females may constantly producing clutches which may be energetically costly to females (G. Garcia. pers. comm.).

A suggested sex ratio of 1:2 is advised although this is an area that requires further research.

2.3.2 Sharing enclosure with other species

There is currently no evidence on the subject of *Alytes* species sharing an enclosure with other members of the genus or with separate species. If any other species are added to the enclosure they should be naturally sympatric with the *Alytes* in their wild state, but due to risk of novel pathogen transmission it is not advised that different species are housed together.

2.4 Breeding

Generally *A. muletensis* and *A. dickhilleni* are bred relatively easily in captivity. *A. obstetricans* prove to be rather erratic breeders under similar environmental and social conditions, as it is thought that *A. obstetricans* require a more pronounced cool period in order to induce breeding. *A. dickhilleni* are managed with an overwinter drop in temperature at Bioparc Fuengirola.

2.4.1 Mating

Captive *A. muletensis* will mate year round in non-seasonal, warm conditions. This is not considered optimal as reproduction is energetically costly. Under natural conditions the animals mate from spring to autumn. Increasing temperatures in spring induces mating. Frequent spraying does increase the activity, but appears to disturb the toads, as does close observation by humans. Captive *A. cisternasii* males have been observed calling from October with first autumn rains, and breed in the winter (S. Bogaerts, pers. comm. 2014). In captive *A. dickhilleni* the first egg clutches are observed from March to June (Garcia-Alonso, pers. comm.).

2.4.2 Pregnancy/ egg laying and incubation

Clutch sizes in captivity will depend on the size, age and nutritional status of the toads. Female *A. muletensis* will drop unfertilised eggs if they cannot find a male to mate with (Bush & Bell, 1997). Males carry the eggs on land until they are ready to hatch, and must be provided with a constant source of water during this time, as they may visit the water in order to keep the eggs moist, and then deposit the eggs in the water when they are ready to hatch (Pinya & Perez-Mellado, 2014). At ZSL London Zoo, data is taken on which males are carrying eggs, when eggs are deposited, and once dropped how many eggs are dead/ mouldy/ failed to hatch, as well as the number of live larvae (Table 6).

It is important to regularly check egg-carrying males for any swellings of the legs as egg strings can constrict their legs just below the knee. If undetected for too long, the leg can become necrotic which will require the amputation of the affected leg or parts thereof. If a swelling is detected, the

egg string needs to be completely removed immediately. The removed eggs can be incubated artificially which can be successful especially in more advanced clutches.



Alytes egg record sheet



Species:
Torrent / Locality:
Year:

Date	Clutch number	Tank	♂	♀	Number of eggs	Drop date	Hatch date	Number of tadpoles	Number of infertile eggs	Number of fertile but not hatched

Table 6. Example breeding record sheet.

In *A. dickhilleni* there is a greater proportion of infertile eggs than is documented in the wild (Garcia-Alonso, pers. comm.).

Egg clutches that are fertile and dropped by the males may be reared on a substrate of moist vermiculite (ratio of water to vermiculite; 1:1 by weight) in a small plastic container. Rotating the eggs regularly and lightly spraying with water is key to the development of the eggs. It is critical to determine when the eggs are ready to hatch; the egg capsule changes and becomes less turgid. At this point the clutch will need to be transferred to water (G. Garcia, pers. comm.).

2.4.3 Use of hormones

The use of artificial hormones to induce breeding has been used with partial success with *A. muletensis* by Matinez-Rica et al. (1984). The use of hormones requires species-specific regimes and may be beneficial in terms of logistics in that synchronised breeding for planned reintroductions can be achieved. However, this strategy should be carefully used since it does have the potential harm to individuals and may mask sub-optimal husbandry practices.

2.4.4 Birth/Hatching

At ZSL London Zoo, once hatched, larvae are left in a separate container for 24 hours to allow them to absorb their yolk reserves. Eggs and larvae are counted, and any infertile eggs and mortalities recorded.

About 86% of all eggs are fertile, and 75% of all eggs laid hatch. Like clutch size, individual fertility increases with age (Tonge & Bloxam, 1991).

2.4.5 Larval development

Once larvae start reabsorbing the tail, they should be offered an environmental gradient from aquatic to terrestrial in order to allow them to emerge. Once emerged and the tail fully absorbed, they should be offered food such as fruit-flies and micro-crickets dusted with Nutrobal; around 5 food items per animal is sufficient. Metamorphs should be offered food more frequently than large juveniles and adults. Newly metamorphosed *A. muletensis* are offered food five times per week at ZSL London Zoo.

2.4.6 Population management

Populations of *A. muletensis* should be kept according to their torrent of origin. This is due to the potential for differences in the genetic makeup of populations who are prevented from dispersing and breeding in the wild state due to physical barriers (e.g. unsuitable habitat, un-scalable waterfalls or man-made obstacles). These populations may have adaptations specific to the microhabitat they originated from, and interbreeding of populations may dilute these adaptations, or produce other deleterious effects associated with hybridisation. This consideration is particularly important when the findings of Kraaijeveld-Smit et al. (2005) are taken into account: wild populations of *A. muletensis* show no dispersal between torrents, and low frequency dispersal between pools in the same torrent which is almost exclusively down-stream.

In conservation breeding programmes it is also important that adaptations to captivity are minimised, as any loss of natural behaviour can be detrimental to the individual and the future of the species as a whole. Tadpoles of the *A. muletensis* maintained in captivity for more than eight generations exhibited a loss of genetic variation and an associated decrease in an anti-predator defensive trait (Kraaijeveld-Smit et al., 2006).

Such adaptations can be avoided by providing conditions as close as possible to those the toads would experience in the wild state.

2.5 Handling

2.5.1 Individual identification and sexing

Identifying male *Alytes* is easiest after mating has occurred, as males will carry the strings of eggs; females may be identified when gravid as a visual inspection of the ventral surface will reveal eggs which are visible.

It may be easier to identify individuals of some *Alytes* species than others; *A. obstetricans* individuals are hard to tell apart visually and without the aid of computer programmes as they have very complex patterns and markings. *A. muletensis* on the other hand are relatively easy to identify from photos as patterns are less complex and can differ markedly between individuals (Fig. 28).

Where individuals cannot be easily distinguished based on morphological traits, they may be artificially marked, for example by using a Visible Implant Elastomer (VIE) which fluoresce under

black light (Fig. 30). In captivity, this method can be also useful to mark cohorts of larvae or identify individuals belonging to the same clutch of eggs. E.g. all the tadpoles with red marking belong to clutch “A” and the ones with blue marking belong to clutch “B” from different years, etc. It is important that marking methods do not compromise the welfare of the individuals, and one advantage of the VIE is that the tags are small and do not displace any tissue (Fig. 29-30). However more study is needed into the direct impact of tagging, and how permanent this method of marking is; evidence by Grant (2010) suggests that such tags do not survive metamorphosis in Wood frogs (*Rana sylvatica*) and maybe lost at metamorphosis in *A. muletensis* if larvae are marked in the tail rather than the dorsum (Fig. 31). VIE is used to identify captive *A. dickhilleni* at Bioparc Fuengirola (Garcia-Alonso, pers. comm.) (Fig. 32).

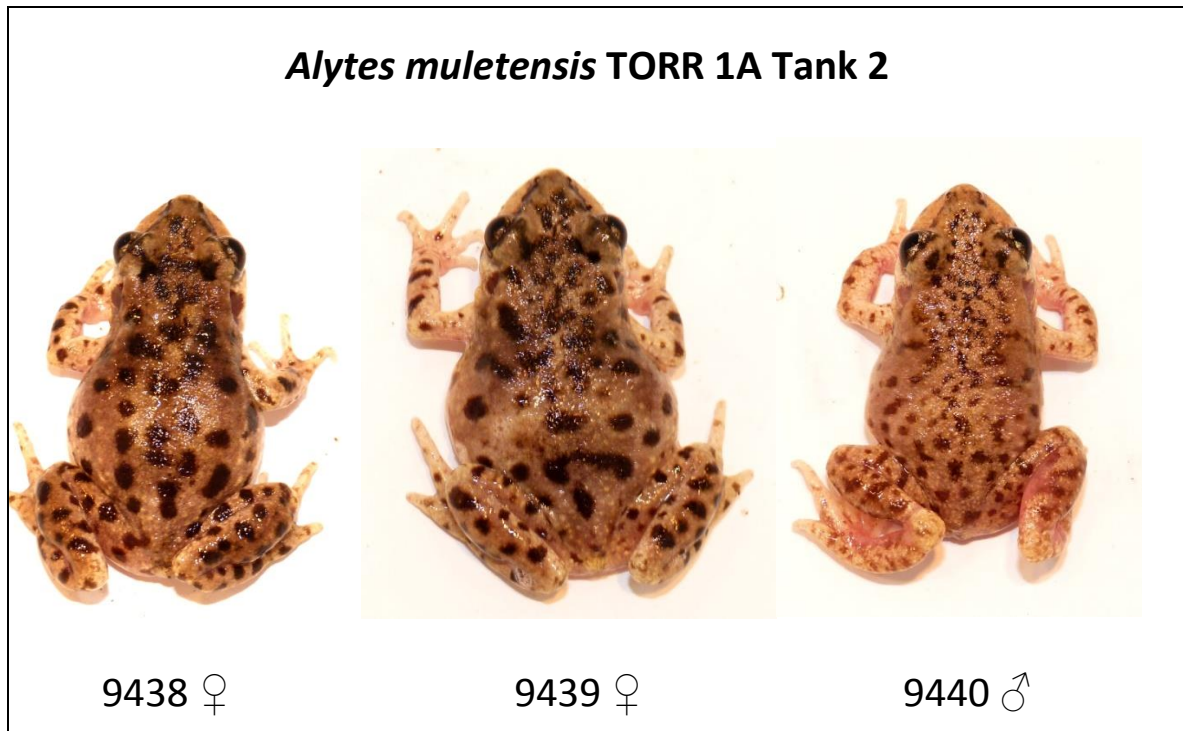


Fig. 28. Identification photos of *A. muletensis* individuals (Dan Lay).



Fig. 29 (Top left). *A. muletensis* larvae marked with VIE (G. Garcia); Fig 30. (Top right) *Alytes muletensis* marked with VIE under a black light (G. Garcia); Fig. 31. (Bottom left) *Alytes muletensis* metamorph with rejected VIE mark (G. Garcia); Fig. 32. (Bottom left) VIE identification of *A. dickhilleni* (D. Garcia-Alonso).

2.5.2 General handling

Handling of *Alytes* should be carried out only if necessary, and handling time should be kept to an absolute minimum, as they are temperate species and can easily over-heat in the hand. When picking them up one should hold individuals loosely in the hand to ensure no damage occurs to any of the toad's limbs. Handlers must wear powder-free nitrile gloves to avoid transmission of harmful pathogens or chemical residue from the hands of the wearer, and gloves should be changed between species or individuals as appropriate; at ZSL London Zoo a service order is followed, with larvae at the start and adults at the end, in order that any spread of pathogens is unidirectional. Gloves are changed if going back in the service order for any reason.

2.5.3 Transportation

If transporting *Alytes* precautions should be taken to keep stress to the individuals to a minimum. Providing stable environmental parameters is important: containers should not be allowed to become too hot, cold or dry. Damp moss should be used as a substrate so that the toads have access to moisture and refugia. Larvae should not be transported unless absolutely necessary as the process may be stressful for aquatic organisms. If larvae do need to be transported, important factors to consider are stocking density, filtration, oxygen content of the water and the amount of nitrogenous waste likely to be produced by the individuals during the transit period. During transport the stocking density should not exceed one individual per litre. Water parameters of the

enclosure at the sending institution should be acquired prior to transport and replicated at the receiving institution. Food grade plastic bags make ideal transportation containers for larvae. Glass or plastic containers should not be used during transportation as the animals can rub against it resulting in injury. Bags should be doubled bag to prevent leakages and the corners of the bag should be taped to ensure that larvae do not become wedged into corners during the transport process. 1/3 of the bag should be filled with water for the source aquarium and the remaining two thirds should be filled with O₂ (do not breathe into the bag, since you will be essentially exhaling CO₂). Bags should be secured with elastic bands or cable ties. The transportation container should be packed within a Styrofoam cooler to prevent sudden changes in temperature and to provide a buffer against temperature extremes. If water temperature needs to be increased for any reason a hot water bottle with cool water can be used. To cool the Styrofoam container one can use freezer icepacks. Take temperature readings before the trip to determine how much of the cooling or heating element to use and for how long it retains its effectiveness. The transportation container should be kept out of direct sunlight. The packing containers should be placed in a rigid outer shipping container. To prevent jarring during transport, crushed newspaper or foam packing chips can be used to support the packing containers within the outer shipping container. It is advisable to avoid shipping if weather forecasts predict very hot or very cold temperatures.

2.5.4 Safety

No considerations are necessary for the health of the keeper.

VETERINARY: CONSIDERATIONS FOR HEALTH AND SAFETY

To prevent the spread of pathogens between enclosures, tanks are serviced in the same order every day to allow the tracing of origin of any infection (larvae first, then juveniles, then adults). Gloves can be changed between enclosures and should be changed between species in multi species facilities. Where to implement glove changes will be dependent on the health status of the group which can be assessed through routine health screening. Any groups showing signs of disease can be moved to the end of the service order.

Newly arriving individuals should be quarantined and kept separate from the current captive populations for a period of three months. During this time new arrivals should be screened for the infectious pathogen *Bd*, and bacteriology and parasitology carried out on faecal samples collected one week apart. If any pathogens of concern or illnesses are discovered in an individual toad the affected toad should be isolated from the rest of the group. It may be necessary to treat all individuals within the population / group it came from as positive. At this point in time there are no reliable anti mortem screening techniques for ranaviruses.

For specimens that are to be used for reintroduction purposes dedicated biosecure facilities are recommended and these facilities should not contain non sympatric species or species originating from cosmopolitan zoo collections. Health status of the wild population should be used as a baseline to avoid inadvertently introducing novel pathogens into the wild when carrying out reintroductions of captive bred or captive reared specimens (Roca et al., 2004).

2.6 Specific problems

Health problems, recorded in captivity (Gerardo Garcia and Javier Lopez, pers. comm. and unpublished records)

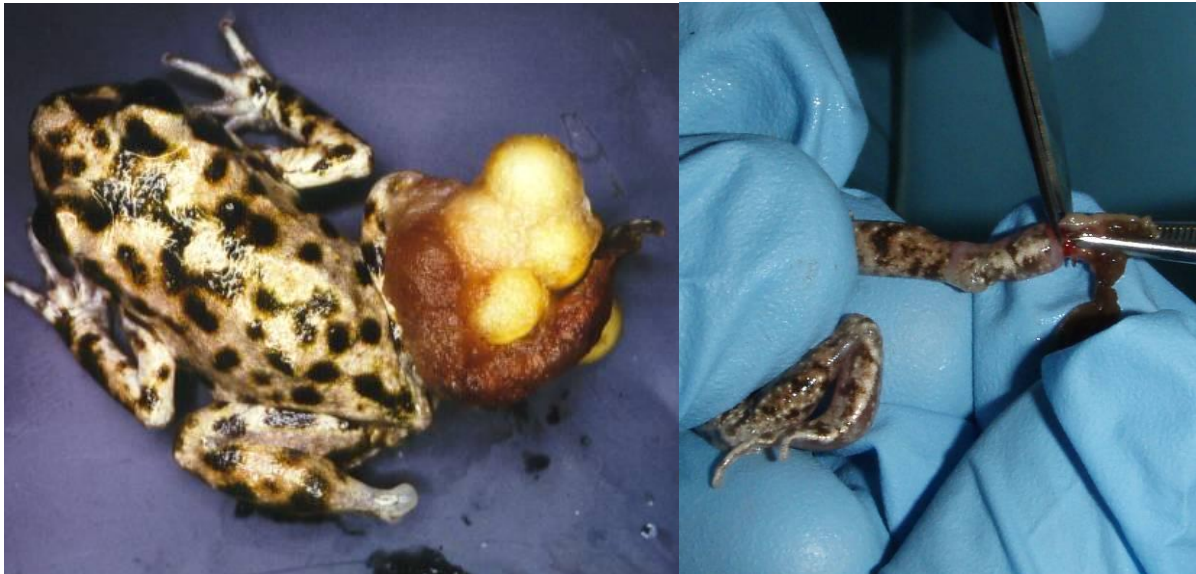


Fig. 33. (Left) Male *Alytes muletensis* with retained egg string and necrotic hind limb (G. Garcia). Fig. 32. (Right) Amputation of necrotic hind limb of *A. muletensis* caused by egg string entanglement (G. Garcia).

- Entanglement of the egg masses around the hind legs of male specimens can result in an annular constriction around the distal aspect of the tibial region. This can cut through the soft tissues of the leg and /or result in the obstruction of the blood flow. If not detected early and treated, the foot swells up and may eventually become necrotic (Fig. 33). Although some specimens can survive after amputation of the foot (Fig. 34), some specimens have died or required euthanasia from this condition. Treatment required removal of the constricting egg mass and antibiotics if there is a wound.
- Mycobacteriosis
- Chytridiomycosis
- Metabolic bone disease
- Egg retention

A number of cases with a distended gall bladder have been observed at post-mortem examination but the significance of this is not clear. It is worth reporting this finding during a PM

2.7 Recommended research

Alytes species are ideal for captive research, as they are relatively well studied, breed well and maintain good health in captive conditions. They therefore provide good candidates for research into genetics, UV provision, infection and treatment of chytrid, and larval nutrition.

Further research into the captive husbandry and ecology of these species is vital in order to support the current amphibian conservation effort, as attention to amphibians has only emerged relatively recently (Odum & Zippel, 2008), and there is relatively little data on amphibians as a group. Amphibians are currently facing a 'global extinction crisis', with many species becoming recently extinct (Zippel et al., 2011). Keeping captive populations for breeding and subsequent re-release into

their natural habitat may be the only option for many extant species, making successful and effective husbandry vital for their conservation.

There are several areas of research for *Alytes* species which require more attention. Firstly, there are little or no available husbandry records for *A. maurus* to the best of our knowledge, and a review of the Zoological Information Management System (ZIMS) and AArk data base indicate that they are not currently part of any official captive program. *Alytes obstetricans* have thus far been problematic to breed in captivity, with the only recorded successful breeding programs housing the toads outside in greenhouses or walled enclosures, to the best of our knowledge (C. Michaels, pers. comm.). Optimal sex ratios in breeding groups and optimal densities for rearing larvae also need to be investigated.

To our knowledge, water parameters at field sites have not been published, this data would be extremely useful for the captive maintenance of *Alytes*.

The conditions required to breed *Alytes* successfully in captivity and in inside enclosures are not currently well known or understood, and may differ for each of the species. Of the species currently known in captivity, *A. cisternasii* and *A. muletensis* both require some form of seasonal cycling to induce breeding. In their natural habitat water availability is subject to seasonal variation, and the climate follows a Mediterranean temperature regime, which is relatively easy to simulate in captivity. If water availability acts as a breeding cue in their natural habitat, the provision of water in captivity may have a similar effect, possibly increasing the ease with which they will breed.

Another area which requires attention is treatment of chytridiomycosis, for which a safer method has been developed for *A. cisternasii* and *A. muletensis*, but not yet for the other species (Martel et al., 2010).

More information is also needed on the wild habits of this genus, as it has been shown that providing conditions as close as possible to those the individuals are accustomed to in the wild state is important for breeding success and the welfare of the animals. In addition to this, successful conservation of *Alytes* requires knowledge of the factors that threaten them. One such factor is predators, such as *N. maura*, and competitors such as *P. perezi*.

Acknowledgements

Special thanks to, Matthias Goetz, Javier Lopez and Christopher Michaels for their valuable assistance and information in the compilation of these guidelines, and to Sergé Bogaerts, Dan Lay, Rafael Marquez, Wouter Beukema, Pierre Fidenci and Miguel Vences for their photos and input.

References

- ALCOVER, J.A., MAYOL, J., JAUME, D., ALOMAR, G. & JURADO, J. (1984) Biología i Ecologia de les poblacions de *Baleaphryne muletensis* a la muntanya mallorquina. In: Història Biològica del Ferreret. Monografies científiques 3, pp. 129-152. Hemmer, H., Alcover, J.A., (eds). Editorial Moll, Palma de Mallorca.
- ANGELIER, E. & ANGELIER, M-L. (1964) Etude d'une population de crapauds accoucheurs dans un lac de haute montagne. *Comptes Rendus de l'Académie des Sciences*. 258: 701-703.
- ANTWIS, R.E. & BROWNE, R.K. (2009) Ultraviolet radiation and vitamin D3 in amphibian health, behaviour, diet and conservation. *Comparative Biochemistry and Physiology A: Comparative Physiology*. 154: 184-190.
- ARNTZEN, J. W. & GARCIA-PARIS, M. (1995) Morphological and allozyme studies of midwife toads (genus *Alytes*), including the description of two new taxa from Spain. *Contributions to Zoology*. 65: 5-34.
- BALL, I. R. (1967) The relative susceptibilities of some species of fresh-water fish to poisons. I. Ammonia. *Water Research*.1: 767–775.
- BARIOS V., OLMEDA C. & RUIZ E. (2012) European Commission Action Plan for the Conservation of the Common Midwife Toad *Alytes obstetricans* in the European Union (2012): Final draft. THE N2K GROUP European Economic Interest Group.
- 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*. In: IUCN 2014. IUCN Red List of Threatened Species. Version 2014.1. <<http://www.iucnredlist.org/details/55266/0>>. Downloaded on 30 June 2014.
- BÖLLET, S., ERBEN, R.G., LINENMAIR, K.E. (1997) Wie zuverlässig ist die skeletochronologische Altersbestimmung bei der Geburtshelferkröte *Alytes obstetricans*? *Mertensiella*. 7:315–327
- 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*. In: IUCN 2014. IUCN Red List of Threatened Species. Version 2014.1. <www.iucnredlist.org>. Downloaded on 30 June 2014.
- BOSCH, J., GARCIA-ALONSO, D., FERNÁNDEZ-BEASKOETXEA, S., FISHER, M.C. & GARNER, T.W. (2013) Evidence for the introduction of Lethal Chytridiomycosis affecting Wild Betic Midwife Toads (*Alytes dickhilleni*). *Eco Health*. 10: 82-89.
- BOSCH, J. and MARQUEZ, R. (1996) Discriminant functions for the sex identification in two midwife toads (*Alytes obstetricans* and *A. cisternasii*). *Herpetological Journal*. 6: 105-109.
- BOSCH, J., MARTÍNEZ-SOLANO, I. & GARCÍA-PARÍS, M. (2001). Evidence of a chytrid fungus infection involved in the decline of the common midwife toad (*Alytes obstetricans*) in protected areas of Central Spain. *Biological Conservation*. 97: 331-337.

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. & PODLOUCKY, R. (2009b) *Alytes dickhilleni*. In: IUCN 2014. IUCN Red List of Threatened Species. Version 2014.1. <www.iucnredlist.org>. Downloaded on 30 June 2014.

BOSCH, J. & GONZÁLEZ-MIRAS, E. (Eds). (2012) *Seguimiento de Alytes dichilleni: Informe final*. Monografías SARE. Asociación Herpetológica Española. Ministerio de Agricultura, Alimentación Y Medio Ambiente. Madrid.

BROWN, L. E. & CRESPO, E. G. (2000) Burrowing behavior of the midwife toads *Alytes cisternasii* and *Alytes obstetricans* (Anura, Discoglossidae). *Alytes*, 17: 101-113.

BROWNE, R.K & ZIPPEL, K. (2007) Reproduction and larval rearing of amphibians. *ILAR Journal*. 48: 214-234.

BRUNGS, W. A. (1971) Chronic effects of low dissolved oxygen concentrations on fathead minnow (*Pimephales promelas*). *Journal of the Fisheries Research Board of Canada*. 31: 1119–1123.

BUCHHOLZ, S. (1989) Untersuchungen zur Fortpflanzungsbiologie und Populationsdynamik einer Freilandpopulation von *Alytes obstetricans* (Amphibia, Anura, Discoglossidae). Diplomarbeit, Universität Würzburg, 81 S.

BUSH, S.L. (1993) Courtship and Male Parental Care in the Mallorcan Midwife Toad, *Alytes muletensis*, Ph.D. thesis, University of East Anglia.

BUSH, S. (1996): Why is double clutching rare in the Majorcan midwife toad? *Animal Behaviour*. 52: 913 – 922.

BUSH, S. L. & BELL, D. J. (1997) Courtship and Female Competition in the Majorcan Midwife Toad, *Alytes muletensis*. *Ethology*. 103: 292–303.

CAMBAR R. & MARTIN, S. (1959) Table chronologique du developpement embryonnaire et lavaire du crapaud accoucheur (*Alytes obstetricans* Laur.). *Act. Soc. Lynn. Bordeaux*. 98: 3-33.

CAMPENY, R. & CASINOS, A. (1989) Densities and buoyancy in tadpoles of midwife toad, *Alytes obstetricans*. *Zoologischer Anzeiger*, 223: 6-12.

CARLSON, A. R. & SIEFERT, R. E. (1974) Effects of reduced oxygen on the embryos and larvae of lake trout (*Salvelinus namaycush*) and largemouth bass (*Micro-Micropterus salmoides*). *Journal of the Fisheries Research Board of Canada*. 31: 1393–1396.

CRESPO, E.G. (1979) Contribuição para o conhecimento da biología das espécies Ibéricas de *Alytes*, *Alytes obstetricans boscai* Lataste (1879) e *A. cisternasii* Bosca (1879): a problemática da especiação de *A. cisternasii*. Unpublished doctoral dissertation. University of Lisboa. In: PINYA, A. and PEREZ-MELLADO, V. (2014): Clutch size in wild populations of *Alytes muletensis*. *Acta Herpetologica*. 9: 115-117.

- CRUZ, M.J., SEGURADO, P., SOUSA, M. & REBELO, R. (2008) Collapse of the amphibian community of the Paul do Boquilobo Natural Reserve (central Portugal) after the arrival of the exotic American crayfish *Procambarus clarkii*. *The Herpetological Journal*. 18: 197-204.
- CUMMINS, C. P. (1989) Interaction between the effects of pH and density on growth and development in *Rana temporaria* L. tadpoles. *Functional Ecology*. 3: 45–52.
- DONAIRE-BARROSO, D. & BOGAERTS, S., (2003) Datos sobre taxonomía, ecología y biología de *Alytes maurus* (PASTEUR and BONS, 1962) (Anura ; Discoglossidae). *Bull. Soc. Cat. Herp.* 16: 25-41.
- DONAIRE-BARROSO, D., EL MOUDEN, E.H., SLIMANI, T. & GONZALEZ DE LA VEGA, J.P. (2006) On the meridional distribution of *Alytes maurus* (PASTEUR and BONS, 1962) (Amphibia, Discoglossidae). *Herpetological Bulletin*. 96: 12-13.
- DONAIRE-BARROSO, D., SALVADOR, A., SLIMANI, T., EL MOUDEN, E.H. & MARTINEZ-SOLANO, I. (2009) *Alytes maurus*. In: IUCN 2014. IUCN Red List of Threatened Species. Version 2014.1. <www.iucnredlist.org>. Downloaded on 30 June 2014.
- EGEA-SERRANO, A., OLIVA-PATERNA, F.J., TEJEDO, M. & TORRALVA, M., (2006) Breeding habitat selection of an endangered species in an arid zone: the case of *Alytes dickhilleni* (ARNTZEN and GARCIA-PARIS, 1995). *Acta Herpetologica*. 1: 81-94.
- ENGELMANN, W.-E., GUENTER, R., & OBST, F. J. (1985) *Lurche und Kriechtiere Europas*. Neumann Verlag, Leipzig. From: (b) Written by Arie Van der Meijden (amphibia AT arievandermeijden.nl), Museum of Vertebrate Zoology, UC Berkeley First submitted 1999-09-22 Edited by VREDENBURG, V. & WHITTAKER, K. (2010-01-18). Available from: http://amphibiaweb.org/cgi/amphib_query?where-genus=Alytes&where-species=obstetricans .Accessed: 13/11/14.
- ENVIRONMENTAL PROTECTION AGENCY (1976) Quality criteria for water, July 1976. Washington, DC: U.S. Environmental Protection Agency. In: ODUM, R.A. & ZIPPEL, K.C., (2008) Amphibian water quality: approaches to an essential environmental parameter. *International Zoo Yearbook*. (42): 40-52.
- EWERT, J. P. & BURHAGEN, H. (1979) Selection by *Bufo*, *Alytes*, *Bombina* and *Hyla*. *Brain Behavior and Evolution*. 16: 157-175.
- GALAN, P., VENCES, M., GLAW, F., FERNADENZ-ARIAS, B. & GARCIA-PARIS, M. (1990) Beobachtungen zur Biologie von *Alytes obstetricans* in Nordwestberien. *Herpetofauna*. 12: 17-24.
- GARCIA-PARIS, M. (1985) Los Anfibios de España. –Ministerio de Agricultura, Pesca y Alimentación, Madrid, Spain. In: MARQUEZ, R., (1992): Terrestrial paternal care and short-term breeding seasons: reproductive phenology of the midwife toads *Alytes obstetricans* and *A. cisternasii*. *Ecography*. 15: 279-288.
- GASCON, C., COLLINS, J.P., MOORE, R.D. et al. (2007) Amphibian conservation action plan. Gland (CH). Cambridge, UK: IUCN/SSC Amphibian Specialist Group.
- GONZALEZ-MIRAS, E., GARCIA-CARDENETE, L. & TEJEDO, M. (2012) Historia Natural. In: BOSCG, J., GONZALEZ-MIRAS, E. (eds). Seguimiento de *Alytes dickhilleni*: Informe final. Monografías SARE.

Asociación Herpetológica Española, Ministerio de Agricultura, Alimentación y Medio Ambiente, Madrid : pp. 17-22.

GONZALEZ-MIRAS, E., GARCIA-CARDENETE, L. & TEJEDO, M. (2012) Historia natural. Pp. 17-22. In: BOSCH, J., GONZALEZ-MIRAS, E. (2012). *Seguimiento de Alytes dickhilleni: Informe final*. Monografías SARE, 02. Asociación Herpetológica Española – Ministerio de Agricultura, Alimentación y Medio Ambiente, Madrid. 84 pp.

GRANY E.H.C. (2010) Visual Implant Elastomer Mark Retention Through Metamorphosis in Amphibian Larvae. *Journal of Wildlife Management*. 72: 1247-1252.

GULIDOV, M. V. (1969) Embryonic development of the pike (*Esox lucius* L.) when incubated under different oxygen conditions. *Problems of Ichthyology*. 9: 841–851.

GUYETENT, R. (1973) Conséquences de l'effet de groupe chez le têtard d'*Alytes obstetricans* Laur. *Zeitschrift für Anatomie und Entwicklungsgeschichte*. 140: 173-186.

HERBERT, D. W. M. & SHURBEN, D. S. (1965) The susceptibility of salmonid fish to poisons under estuarine conditions. II. Ammonium chloride. *International Journal of Air and Water Pollution*. 9: 89–91.

JOFRE, M. B. & KARASOV, W. H. (1999) Direct effect of ammonia on three species of North American anuran amphibians. *Environmental Toxicology and Chemistry*. 18: 1806–1812.

KLINGLER, K. (1957) Sodium nitrate, a slow acting fish poison. *Schweizerische Zeitschrift fuer Hydrologie*. 19: 565–578.

KRAAIJEVELD-SMIT, F. J. L., BEEBEE, T. J. C., GRIFFITHS, R. A., MOORE, R. D., & SCHLEY, L. (2005) Low gene flow but high genetic diversity in the threatened Mallorcan midwife toad *Alytes muletensis*. *Molecular Ecology*. 14: 3307-3315.

KRAAIJEVELD-SMIT, F.J.L., GRIFFITHS, R. A., MOORE, R.D. & BEEBEE, T.J.C. (2006) Captive breeding and the fitness of reintroduced species: a test of the response to predators in a threatened amphibian. *Journal of Applied Ecology*. 43: 360-365.

LEA, J., MANDY, D. & HALLIDAY, T. (2002a) The effects of cohort structure and density on larval growth and development in *Alytes muletensis*: Implications for conservation. *Herpetological Journal*. 12: 155-161.

LEA, J., MANDY, D. & HALLIDAY, T. (2002b) Phonotaxis advertisement calls by midwife toads (*Alytes muletensis*) is not necessarily related to mating. *Amphibia-Reptilia*. 23: 151-159.

LIZANA. M., Ciudad Pizarro, M. J. & Pérez-Mellado, V. (1986) Uso de los recursos tróficos de una comunidad ibérica de anfibios. *Revista Española de Herpetología*. 1: 207-272.

LOPEZ-JURADO, L.F., CABALLERO M.R., & DOS-SANTOS FREITAS, L. (1979) Biología de la reproducción de *Alytes cisternasii* Bosca, 1879. *Donana Acta Vertebrata*. 61: 5-17.

MALKMUS, R. (1983) Zur Fortpflanzungsbiologie von *A. cisternasii*. *Weinstadt*. 5: 30-34.

- MARCO, A., QUICHANO, C. & BLAUSTEIN, A. R. (1999) Sensitivity to nitrate and nitrite in pond-breeding amphibians from the Pacific Northwest, USA. *Environmental Toxicology and Chemistry*. 18: 2836–2839.
- MARQUEZ, R., BELTRAN, J.F., SLIMANI, T., RADI, M., LLUSIA, D., & EL MOUDEN, E.H., (2011) Description of the advertisement call of the Moroccan Midwife Toad (*Alytes maurus* PASTEUR and BONS, 1962). *Alytes*. 27: 142-150.
- MARQUEZ, 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.
- MARQUEZ, R. & BOSCH, J. (1996) Advertisement call of the midwife toad from the Sierras Béticas *Alytes dickhilleni* (Amphibia, Anura, Discoglossidae). *Herpetological Journal*. 6: 9-14.
- 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.
- MARQUEZ, R. (1992) Terrestrial paternal care and short-term breeding seasons: reproductive phenology of the midwife toads *Alytes obstetricans* and *A. cisternasii*. *Ecography*. 15: 279-288.
- MARTEL, A., VAN ROOIJ, P., VERCAUTEREN, G., BAERT, K., VAN WAEYENBERGHE, L., DEBACKER, P., GARNER, T.W. J., WOELTJES, T., DUCATELLE, R., HAESBROUCK, F. & PASMANS, F. (2010) Developing a safe antifungal treatment protocol to eliminate *Batrachochytrium dendrobatidis* from amphibians. *Medical Mycology Month*. 49: 1-7.
- MARTÍNEZ-RICA J.P., PARDO, M.P. & CERVANTES, J. (1984) La reproducción y la conducta en cautividad del sapillo balear, *Baleophryne muletensis*. In: *Història Biològica del Ferreret*. Hemmer, H. and Alcover, J.A. (Eds). Editorial Moll, Palma de Mallorca, Spain. 3: 175-191.
- 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: Discoglossidae). *Herpetological Journal*. 13: 89-94.
- MARTINS, F., 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.
- MCDIARMID, R.W. & ALTIG, R. (eds.) (1999) *Tadpoles: The Biology of Anuran Larvae*. University of Chicago Press, Chicago, USA.
- MELLADO, J. (1976) Sobre la alimentación del sapo partero ibérico (*Alytes cisternasii* Boscá) (Anura, Discoglossidae). *Boletín de la Estación Central de Ecología*. 5: 81-84.
- 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.
- MOORE, R.D., GRIFFITHS, R.A. & ROMAN, A. (2004) Distribution of the Mallorcan midwife toad (*Alytes muletensis*) in relation to landscape topography and introduced predators. *Biological Conservation*. 116: 327-332.

- MOU, Y. P. (1985) Alimentation d'une population d'*Alytes obstetricans* dans l'ouest de la France. *Alytes*. 4: 147-152.
- NÖLLERT, A. & NÖLLERT, C. (1992) Die Amphibien Europas. Franckh-Kosmos Verlags-GmbH and Company, Stuttgart. From: Written by VAN DER MEIJDEN, A., CAVAGNARO, J. (amphibia AT arievandermeijden.nl), Research associate, Museum of Vertebrate Zoology, UC Berkeley
First submitted 1999-09-22 Edited by Vance Vredenburg (2012-04-03). Available at:
http://amphibiaweb.org/cgi/amphib_query?where-genus=Alytes&where-species=dickhilleni
.Accessed: 13/11/14.
- OLIVIER, J. A. (2014) Ferreret – *Alytes muletensis*.in: *Enciclopedia Virtual de los Vertebrados Españoles*. SALVADOR, A., MARTINEZ-SOLANO, I. (eds.). Museo Nacional de Ciencias Naturales, Madrid
- ODUM, R. A., MCCLAIN, J. M. & SHELY, T. C. (1984) Hormonally induced breeding and rearing of white's tree frog, *Litoria caerulea* (Anura: Pelodyadidae). In: Proceedings 7th reptile symposium on captive propagation husbandry. Tolson, P. J. (Ed.). (1983) Pp.42–52. Thurmont, MD: Zoological Consortium.
- ODUM, R.A. & ZIPPEL, K.C. (2008) Amphibian water quality: approaches to an essential environmental parameter. *International Zoo Yearbook*. 42: 40-52.
- PASTEUR, G. & J. BONS. (1962) Note préliminaire sur *Alytes maurus*: gemellarité ou polytopisme? Remarques biogéographiques, génétiques et taxinomiques. *Bulletin de la Société Zoologique de France*. 87: 71–79.
- PINYA, S. & PÉREZ-MELLADO, V. (2013) Ageing and growth of the endangered midwife toad *Alytes muletensis*. *Endangered Species Research*. 22: 263-268.
- PINYA, A. & PEREZ-MELLADO, V. (2014) Clutch size in wild populations of *Alytes muletensis*. *Acta Herpetologica*. 9: 115-117.
- PRICE, S.J., GARNER, T.W.J., NICHOLS, R.A., BALLOUX, F., AYRES, C., DE ALBA, A.M.C. & BOSCH, J. (2014): Collapse of amphibian communities due to an introduced *Ranavirus*. *Current Biology*. <http://dx.doi.org/10.1016/j.cub.2014.09.028>.
- PRYOR, G.S. (2014) Tadpole nutritional ecology and digestive physiology: implications for captive rearing of larval anurans. *Zoo Biology*. 33: 502-507.
- READING, C.J. & CLARKE, T. (1988) Multiple clutches, egg mortality and mate choice in the midwife toad, *Alytes obstetricans*. *Amphibia-Reptilia*. 9: 357-364.
- ROCA, V., GALDÓN, M.A., MARTIN, J.E., GARCIA, G. & LOPEZ, J. (2004) Primeros datos acerca de la parasitación de la población natural del sapillo Balear, *Alytes muletensis* (SANCHIZ et ADROVER, 1977) (Anura: Discoglossidae). *Boletín de la Asociación Herpetológica Española*. 15: 44-49.
- RODRIGUEZ JIMENEZ, A.J. (1984) Fenología del sapo partero iberico (*Alytes cisternasii* Bosca. 1879). *Alytes* (España) 2: 9-23.

- ROMAN, A. & SERRA, J.M. (1997) La recuperacion del ferreret, *Alytes muletensis*. Documents tecnicos de Conservacio 1. Conselleria de Medi Ambient, Ordenacio del Territori j Litoral, Palma de Mallorca. In :PINYA, A. and PEREZ-MELLADO, V.(2014): Clutch size in wild populations of *Alytes muletensis*. *Acta Herpetologica*. 9: 115-117.
- ROSA, G.M., ANZA, I., MOREIRA, P.L., CONDE, J., MARTINS, F., FISHER, M.C. & BOSCH, J. (2013) Evidence of chytrid-mediated population declines in common midwife toad in Serra da Estrela, Portugal. *Animal Conservation*. 16: 306-315
- ROUSE, J. D., BISHOP, C.A. & STRUGER, J. (1999) Nitrogen pollution: an assessment of it threats to amphibian survival. *Environmental Health Perspectives*. 107: 799–803.
- RUSSO, R. C., SMITH, C. E. & THURSTON, R. V. (1974) Acute toxicity of nitrite to rainbow trout (*Salmo gairdneri*). *Journal of the Fisheries Research Board of Canada*. 31: 1653–1655.
- SCHLEICH, H. H., KASTLE, W. & KABISCH, K. (1996) Amphibians and Reptiles of North Africa- Biology, Systematics, Field Guide. Koeltz Scientific Publishers, Koenigstein. 630pp.
- SCHLEY, L. & GRIFFITHS, R.A., (1997) Midwife Toads (*Alytes muletensis*) avoid chemical cues from snakes (*Natrix maura*). *Journal of Herpetology*. 32: 572-574.
- SCHLEY, L., GRIFFITHS, R.A. & ROMÁN, A. (1998) Activity patterns and microhabitat selection of Mallorcan midwife toad (*Alytes muletensis*) tadpoles in natural torrent pools. *Amphibia-Reptilia*. 19: 143-151.
- SERRA, J.M., GRIFFITHS, R., BOSCH, J.,BEEBEE, T., SCHMIDT, B.,TEJEDO, M.,LIZANA, M.,MARTÍNEZ-SOLANO, I.,SALVADOR, A.,GARCÍA-PARÍS, M.,GIL, E.R. & ARNTZEN, J.W. (2009) *Alytes muletensis*. In: IUCN 2014. IUCN Red List of Threatened Species. Version 2014.1. <www.iucnredlist.org>. Downloaded on 30 June 2014.
- SIEFERT, R. E., CARLSON, A. R. & HERMAN, L. J. (1975) Effects of reduced oxygen concentrations on the early life stages of mountain whitefish, smallmouth bass, and white bass. *Progressive Fish-Culturist*. 36: 186–190.
- SIEFERT, R. E. & SPOOR, W. A. (1974) Effects of reduced oxygen on embryos and larvae of white sucker, coho salmon, brook trout and walleye. In: The early life history of fish, BLAXTER, J. H. S. (Ed.). Springer Berlin Heidelberg, Berlin. Pp.487–495.
- SOARES, C., ALVES DE MATOS, A., ARNTZEN, J.W., CARRETERO, M., LOUREIRO, A. (2003). Amphibian mortality in a National Park in the North of Portugal. *FrogLog*. 56: 1
- SPENCE, D. (2002) A comparison of fitness in founder and descent stock of Mallorcan midwife toad (*Alytes muletensis*) tadpoles, MSc thesis, Durrell Inst. of Conservation and Ecology, Univ. Kent, Canterbury.
- TABATA, K. (1962) Toxicity of ammonia to aquatic animals with reference to the effect of pH and carbon dioxide. *Bulletin of the Tokai Regional Fisheries Research Laboratory*. 34: 67–74.

TAPLEY, B., RENDLE, M., BAINES, F.M., GOETZ, M., BRADFIELD, K.S., ROOD, D., LOPEZ, J., GARCIA, G., & ROUTH, A. (2015) 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*. 34: 46-52.

TONGE, S. & BLOXAM, Q. (1989) Breeding the Mallorcan midwife toad *Alytes muletensis* in captivity. *International Zoo Yearbook*. 28: 45 -53.

TONGE, S.J. and BLOXAM, Q.M.C. (1991) The breeding programme for the Mallorcan Midwife Toad *Alytes muletensis* at the Jersey Wildlife Preservation Trust. *Dodo, Journal of the Jersey Wildlife Preservation Trust*. 27: 146 – 156.

VIERTEL, B. (1984) Suspension feeding of the larvae of *Baleaphryne muletensis*. Pp. 153-161. En: HEMMER, H. & ALCOVER, J.A. (Eds.). *Història Biològica del Ferreret (Life History of the Mallorcan Midwife Toad)*. Ed. Moll, Palma de Mallorca.

WALKER, S.F., BOSCH, J., JAMES, T.Y., LITVINTSEVA, A.P., VALLS, J.A.O., PIÑA, S., GARCÍA, G., ROSA, G.A., CUNNINGHAM, A.A., S. HOLE, GRIFFITHS, R. & FISHER, M.C. (2008) Invasive pathogens threaten species recovery programs. *Current Biology*. 18: 1-2.

WARNER, S.C., DUNSON, W. A. & TRAVIS, J. (1991) Interaction of pH, density, and priority effects on the survivorship and growth of two species of hyloid tadpoles. *Oecologia*. 88: 331–339.

WELLS, K.D. (2007) The ecology and behaviour of amphibians. The University of Chicago Press: Chicago. 1148pp.

WESTIN, D. T. (1974) Nitrate and nitrite toxicity to salmonid fishes. *Progressive Fish-Culturist*. 36: 86–89.

WHITAKER, B. R. (2001) Water quality. In: Amphibian medicine and captive husbandry. Wright, K.M. & Whitaker, B. R. (Eds). Malabar, FL: Krieger Publishing. 147–157.

ZIEGLER, N. (2000): Mallorcan Midwife Toad (*Alytes muletensis*), Species Management Guidelines No. 9, Durrell Wildlife Conservation Trust.

ZIPPEL, K., JOHNSON, K., GAGLIARDO, R., GIBSON, R., MCFADDEN, M., BROWNE, R., MARTINEZ, C. & TOWNSEND, E. (2011) The Amphibian Ark: A Global Community for Ex Situ Conservation of Amphibians. *Herpetological Conservation and Biology*. 6:340–352.

WEBSITES:

DIETTERICH, L. *Alytes maurus*. Available at: http://amphibiaweb.org/cgi/amphib_query?where-genus=Alytes&where-species=maurus. Accessed: 29/09/14.

EDGEa. 940. Common midwife toad (*Alytes obstetricans*).EDGE of Existence program, Zoological Society London. Available at: http://www.edgeofexistence.org/amphibians/species_info.php?id=1374. Accessed: 29/09/14

EDGEb. 275. "maurus" midwife toad (*Alytes maurus*). EDGE of Existence program, Zoological Society London. Available at: http://www.edgeofexistence.org/amphibians/species_info.php?id=1373. Accessed 29/09/14.

EDGEc. 56. Mallorcan midwife toad (*Alytes muletensis*).EDGE of Existence program, Zoological Society London. Available at: http://www.edgeofexistence.org/amphibians/species_info.php?id=600. Accessed 29/09/14.

FIDENCI, P. (2011) *Alytes obstetricans* photo available at: http://calphotos.berkeley.edu/cgi/img_query?enlarge=0000+0000+0811+3496

VAN DER MEIJDEN, A. & CAVAGNARO, J. (amphibian@arievandermeijden.nl) (1999): *Alytes dickhilleni*. VREDENBURG, V. Ed. (2012). Available at: http://amphibiaweb.org/cgi/amphib_query?where-genus=Alytes&where-species=dickhilleni. Accessed: 29/09/14.

VAN DER MEIJDEN, A. (amphibian@arievandermeijden.nl). (1999a): *Alytes cisternasii*. VREDENBURG, V. and MAHONEY, M. Eds. (2002). Available at: http://amphibiaweb.org/cgi-bin/amphib_query?where-scientific_name=Alytes+cisternasii. Accessed: 29/09/14.

VAN DER MEIJDEN, A. (amphibian@arievandermeijden.nl). (1999b): *Alytes obstetricans*. VREDENBURG, V. and WHITTAKER, K. Eds. (2010). Available at: http://amphibiaweb.org/cgi/amphib_query?where-genus=Alytes&where-species=obstetricans. Accessed: 29/09/14.

VAN DER MEIJDEN, A. (amphibian@arievandermeijden.nl). (1999c): *Alytes muletensis*. VREDENBURG, V. Ed. (2006). Available at: http://www.amphibiaweb.org/cgi/amphib_query?where-genus=Alytes&where-species=muletensis. Accessed: 29/09/14.

WAZA. Common Midwife Toad (*Alytes obstetricans*). World Association of Zoos and Aquariums. Available at: <http://www.waza.org/en/zoo/choose-a-species/amphibians/frogs-and-toads/alytes-obstetricans>. Accessed: 29/09/14.

RAUK 2014. Reptiles and Amphibians of the UK, Midwife Toad - *Alytes obstetricans* – Alien. http://www.herpetofauna.co.uk/midwife_toad.htm

FURTHER READING

BULEY, K.B. & GARCIA, G. (1997) The recovery programme for the Mallorcan Midwife Toad *Alytes muletensis*: an update. *Dodo, Journal of the Jersey Wildlife Preservation Trust*. 33: 80 – 90.

GIBSON, R. (2003) Mallorcan midwife toad (*Alytes muletensis*). Available at: <http://www.arkive.org/mallorcan-midwife-toad/alytes-muletensis/>. Accessed: 29/09/14. Authentication: GIBSON, R., Curator of Herpetology, Zoological Society of London.

GRIFFITHS, R.A., GARCIA, G. & OLIVER, J. (2008) Re-introduction of the Mallorcan midwife toad, Mallorca, Spain. (Eds). SOORAE, P.S. *Global Reintroduction Perspectives*.