

## Clutch size in wild populations of *Alytes muletensis*

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**Abstract.** Clutch size of genus *Alytes* presents strong inter- and intra-specific variability. The Mallorcan midwife toad (*A. muletensis*) is a threatened endemism of the island of Mallorca (Spain), and has the smallest clutch size of the genus. Most of the previously published clutch size information was obtained from animals breeding in captivity. In this study we analysed a large number of clutches from wild populations, and we compared their size (number of eggs) with previously published data from both captive and wild populations. Our results showed that clutch sizes of wild males carrying a single clutch were similar to those observed in captivity. However, multiple clutches were more common in the wild than in captivity.

**Keywords.** *Alytes muletensis*, clutch size, wild populations, endemic species, Balearic Islands

In *Alytes* species it is males that care for eggs. The female expels a strand of eggs which are fertilised by the male before he wraps them around his legs to protect them from predators. When the eggs are ready to hatch, the male wades into shallow ponds or streams to allow tadpoles to go into the water (Stebbins and Cohen, 1997).

Five separate species of midwife toads are found across Western Europe, Northern Africa, and the island of Mallorca: *Alytes obstetricans*, *Alytes cisternasii*, *Alytes dickhilleni*, *Alytes maurus* and *Alytes muletensis* (Martinez-Solano et al., 2004). The clutch size of the genus *Alytes* is highly variable both, inter- and intra-specifically (Table 1). The Mallorcan midwife toad, *A. muletensis*, is a threatened endemism of the island of Mallorca (Balearic Islands, Spain; Serra et al., 2009), with the smallest clutch size of the genus (Alcover et al., 1984, Fig. 1).

As in other *Alytes* species, the clutch size of *A. muletensis* is fairly variable (Table 2). During studies in captivity Bush (1993, 1996) observed the presence of double clutches for the first time in *A. muletensis*, that is, one male carrying the eggs released by two females. He also found that single clutches (less than 15 eggs) had an aver-

age of 10.00 eggs (range from 4 to 14 eggs, Bush, 1993, 1996). Taking into account double clutches, the average increased to 11.36 eggs (range from 4 to 27; Bush, 1996). Additionally, and also in captivity, the maximum clutch size observed by Román and Mayol (1997) had 34 eggs, while the average clutch size was 11 eggs. In order to establish demographic parameters of wild *A. muletensis* individuals, between 2007-2010, we counted the number of eggs carried by 92 males from 11 natural, plus 7 artificial, breeding sites in the wild. An artificial breeding site for the purpose of this study is a man-made structure such as drinking ponds for cattle or old water cisterns/tanks being used by wild *A. muletensis* for breeding. These sites were established as a conservation measure for the release of captive-bred animals during the 1990s. The current sample represents the descendants of these introduced toads. On the other hand, streams and isolated natural pools are considered natural breeding sites. The clutch size was determined by counting the number of eggs of empty strings and egg capsules after the tadpoles had emerged, and within a short time after the males had come out of the water.

**Table 1.** Clutch sizes of continental *Alytes* species. Average eggs per clutch, minimum and maximum eggs per clutch, and source of information.

Species	Average	Range	Reference
<i>A. obstetricans</i>	63	35-95	Crespo, 1979
	104	46-143	López-Jurado et al., 1979
	51	24-142	Buchholz, 1989
	38	5-120	Galán et al., 1990
	27	21-33	Galán et al., 1990
	55	24-77	Galán et al., 1990
	34	6-53	Galán et al., 1990
<i>A. cisternasii</i>	77	32-171	Reading, Clarke, 1998
	73	42-119	Crespo, 1979
	104	46-143	López-Jurado et al., 1979
<i>A. dickhilleni</i>	73	48-116	Malkmus, 1983
	87	20-180	Marquez, 1989
	74	28-149	González-Miras, García-Cardenete & Tejedo, 2012
<i>A. maurus</i>	-	60-70	Donaire-Barroso, Bogaerts, 2003

**Table 2.** Clutch size in *A. muletensis*: summary of literature and original (present study) data. Population type, average clutch size, sample size (when available), minimum and maximum eggs per clutch, and source of information.

Population type	Average	N	Range	Variance	Reference
Natural	12.04	92	4-34	32.53	<b>Present study</b>
Natural	9.78	9	7-12	-	Alcover et. Al, 1984
Captive	-	-	10-24	-	Martínez-Rica et al., 1984.
Captive	11.43	-	9-15	-	Tonge & Bloxam, 1989
Captive	11.36	220	4-27	14.05	Bush, 1996
Captive and Natural	11	-	-34	-	Román & Mayol, 1997

To show clutch size variability in wild populations of *A. muletensis*, descriptive statistics are provided (Table 2). We used one way ANOVA to compare (1) clutch size data from Alcover et al. (1984) with that from the present study, (2) clutch size data from Bush (1996; captive populations) with that from the present study, and (3) clutch size data for artificial and natural breeding sites from the present study. A chi-square test was used to compare frequencies of multiple clutches in captive (from Bush, 1996) and wild (from the present study) populations of *A. muletensis*. All statistical analyses were carried out with Statistica (ver. 6.0).

Our data revealed an average clutch size of 12.04 eggs in the wild (Table 2). We found no significant dif-



**Fig. 1.** Male *A. muletensis* carrying eggs (island of Mallorca, Spain).

ference ( $F_{1,99} = 1.40$ ,  $p = 0.24$ ) when our data was compared to previously published averages for wild individuals (Alcover et al., 1984), maybe because of the small sample size in the 1984 study. There was also no significant difference between clutch size data in our study ( $F_{1,319} = 0.83$ ,  $p = 0.36$ ) and that published by Bush (1996) for animals in captivity. Additionally, there was no significant difference between natural ( $n=41$ ) and artificial ( $n=51$ ) breeding sites in the present study ( $F_{1,92} = 1.31$ ,  $p = 0.26$ ). The observed range of clutch size found in this study is the largest known for both wild and captive populations, and coincides with the minimum and maximum values published previously (Alcover et al., 1984; Bush, 1996; Román and Mayol, 1997).

Multiple clutches are common in natural populations of continental midwife toads (Bush, 1996). Between 56-61 % of *A. obstetricans* and 80 % of *A. cisternasii* egg-carrying males carry two or more clutches simultaneously (Reading and Clarke, 1988; Márquez 1990). In *A. muletensis*, double clutches are rare (12-14 % according to Bush, 1996). On the other hand, triple clutches, those with more than 28 eggs, were first reported by Román and Mayol, (1997) with a record of 34 eggs, but their frequency was not provided. Our data shows that, in wild populations 23.91 % of clutches should be considered multiple clutches, with 3.26 % of them consisting of triple clutches.

When comparing the occurrence of multiple clutches, between wild individuals in this study and data for captive individuals (Bush, 1996), we found a significantly higher frequency of multiple clutches in the wild population ( $\chi^2 = 5.30$ ,  $p < 0.02$ ). The low incidence of multiple clutches in captivity may be due to the short time avail-

able for a male to obtain the second clutch from a female (Bush, 1996). Males in wild populations may have longer time intervals during which they can accept additional clutches. In addition, the operational sex-ratio during the breeding season also determines the potential for multiple clutches. If more females are available males will be able to mate with more than one female in a short period of time and hence brood more than one clutch (Bush, 1996). It is also possible that wild individuals breed at higher densities than captive individuals, and wild receptive males may be able to obtain a second clutch more frequently simply because they are more likely to encounter more than one gravid female.

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