Natural incidence of body abnormalities in the Montseny newt, *Calotriton arnoldi* Carranza and Amat, 2005

Albert Martínez-Silvestre¹, Fèlix Amat² and Salvador Carranza³

Introduction

The decline and wealth of amphibian populations is a recurrent centre of interest in the herpetological scientific community since the past eight decades. One of the most worrisome phenomena is the spread of morphological malformations, especially in frogs, but also occasionally in salamanders (Sequeira et al., 1999; Wheeler, McCallum and Trauth, 2002; Ferrer and Lopez, 2003; Miller and Miller, 2005).

The Montseny newt (Calotriton arnoldi) is one of the most recent described amphibian species in Europe (Carranza and Amat, 2005). Restricted to a small range within the Montseny massif (north eastern Iberian Peninsula), only seven populations structured into two separated areas by a river (Tordera river), are actually known. Population in these two areas, divided in eastern and western, are genetic and morphologically well differentiated (Valbuena-Ureña, Amat and Carranza, 2013). Calotriton asper is an entirely aquatic species, living in fast flowing steams in forestal areas from 650 m to 1.200 m.a.s.l. In the course of conservation surveys carried out in the Natural Park of Montseny, we detected some individuals affected by limb abnormalities in one population of C. arnoldi. Consequently we performed an especific survey to characterize and quantify the incidence of body abnormalities on C. arnoldi.

Material and methods

Field sampling was performed during nine nights, from 17 April to 1 May of 2007, in the eastern population and five nights, from 16 to 26 April of the same year, in the western one.

We examined 98 adults and nine juveniles from the eastern population and 33 adults and 13 juveniles from the western populations (153 newts in total). Newts were caught by hand, sexed, and SVL (snout-vent length) measured by means a digital calliper to 0.1 mm of precision. Before releasing them, newts were marked by means of visible implant elastomere (Northwest Marine Technology, Inc.) to avoid sampling repeatedly the same individuals. Newts were sexed based in the presence of secondary sexual characters (morphology of cloacal area) and considered as adults. We visually examined all the individuals recording the presence of body abnormalities as well as the presence or not of melanophoroma already described in eastern populations (Martínez-Silvestre et al., 2011).

We tested for SVL differences between newts with limb abnormalities and normal ones using a two-way ANOVA test. Age class and sexual effects on the presence on this pathology, and positive association between limb and skin abnormalities in the eastern population, were also tested, by means of Chi-square test.

Results

The total incidence of abnormalities was of 3.92 %. Frequencies of limb malformations were not significant different between the two studied populations (Chi-square test=0.249; d.f.=1; P=0.6177). Limb abnormality was found in the 2.61 % of field-caught adult newts, with a partial result of 75 % of polydactyly and 25% of ectrodactyly (Fig. 1). No abnormalities were found in juveniles. We did not find differences in limb abnormality frequencies between sexes (Chi-square test=1.298; d.f.=1; P=0.2878) and SVL between affected and normal

¹Catalonian Reptile and Amphibian rehabilitation Center (CRARC), 08783, Masquefa, Barcelona, Spain.

² Àrea d'Herpetologia, Museu de Granollers – Ciències Naturals, Francesc Macià 51, 08400, Granollers, Spain.

³Institute of Evolutionary Biology (CSIC-UPF), Passeig Marítim de la Barceloneta 37-49, E-08003, Barcelona, Spain.

Corresponding author; e-mail: crarc@amasquefa.com



Figure 1. A) Detail of polydactyly in the left rear leg of an adult Montseny newt (*Calotriton arnoldii*). B) Adult female with a bifid tail.

newts (ANOVA test: F1 106=0.6066, P=0.4377). On the other hand, limb abnormality occurrence was not related to melanophoroma presence (Chi-square test = 0.6603; d.f.=1; P<0.4164). We only detect an adult female, with a bifid tail (Fig. 2), representing a body abnormality incidence of 0.007 %.

Discussion

We report the evidence of limb abnormality in two wild populations of *C. arnoldi*. The extreme sensibility of amphibians to the environmental changes and the degradation of their habitats (Semlitsch, 2003) put special importance on investigation of amphibian malformations and diseases. A measure of population health is the frequency of body abnormalities.

It is stated that background abnormality rates in amphibians should not exceed 5% in wealthy populations (Meyer-Rochow and Asahima, 1988). The observed incidence of body malformations in the most affected population of *C. arnoldi*, is lower than this threshold. In comparison, with other European salamanders and newts living in apparently not altered habitats, our prevalences are slightly higher, but less than observed in the American ones (Table 1).

Body abnormalities as supernumerary or missing limbs, tails or digits, lack of eyes, macrocephalia and pigmentary anomalies have been related to parasite infection, hyperregeneration, predation. UV-B radiation, exposition to higher environmental temperatures, genetic phenomena and wounds due to intraspecific confrontation (Dearlove and Dresden, 1976; Sessions and Ruth, 1990; Burgmeier et al., 2011). Nevertheless, we did not find traces of water pollution in a chemical analysis carried out in the seven streams of C. arnoldi range. In addition, water composition does not differ between the streams inhabited by the affected populations. On the other hand the main affected population has a high mitochondrial variability in comparison to the other population (Valbuena-Ureña,

Table 1. Relative incidence of be	dy abnormalities in Urodela.
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Species	Frequency	Reference
Lissotriton Boscai	1.1%	Ortiz et al. (2006)
Chioglosa lusitanica	1.5%	Sequeira et al. (1999)
Calotriton arnoldii	3.92 %	This work
Ambystoma macrodactylum	4.7 – 12.5%	Johnson et al. (2006)
Cryptobranchus alleganiensis	40 - 90%	Hiler, Wheeler and Trauth, (2005); Burgmeier et al. (2011)

Amat and Carranza, 2013). Thus, our data do not support a genetic bottleneck association with the spread of abnormalities in this population.

Although the presence of skin abnormalities indicates the action of some environmental stressor (Martínez-Silvestre et al., 2011) they are decoupled from the presence of limb malformations. This reinforces the lack of environmental causation on limb malformations in *C. arnoldi*. Our field observations, indicates that predators are rare in the newt's habitats and the only source of traumatic body amputations provide from the observed aggressive intraspecific interactions, especially among males. This behaviour could contribute in some importance to the limb or tail regeneration and presence of malformations in these newt populations.

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