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Body Malformations in a Forest-dwelling Salamander, *Salamandrina perspicillata* (Savi, 1821)

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***Abstract*.—High percentages of body malformations are considered auxiliary indicators of global amphibian decline. However, information on their frequency in natural populations are rarely provided and sample sizes are often small, particularly for newts and salamanders. In this study we report on the malformations of a population of the Spectacled Salamander (*Salamandrina perspicillata*). We sampled 508 salamanders and assigned body malformations to four main categories. We found one salamander with a bifid tail, an extremely rare abnormality among urodeles, and three types of limb malformations in slightly more than 8% of the salamanders. Females were significantly more malformed than males ($P < 0.05$) and the three limb abnormalities differed significantly in their prevalence, both when pooling all salamanders and when considering sexes separately ($P < 0.001$ in all comparisons). The percentage of malformed individuals largely exceeded what is expected in healthy populations. However, the study site was characterized by low anthropogenic pressure. We suggest some potential causes of the observed malformations including massive trematode parasites infections and the trampling of ungulates that may cause severe injuries on these small salamanders.**

Key Words.—amphibian malformations; bifid tail; brachydactyly; ectrodactyly; Italy; limb abnormalities; polydactyly

Introduction

Population declines and extinctions are normal processes of evolutionary mechanisms. However, ecosystem alterations by human activities have caused a rapid acceleration of extinction processes in many species, severely damaging biodiversity. Among vertebrates, amphibians suffer more than other taxa to such degradation (Stuart et al. 2008). Global amphibian decline has been documented since the early 1990s (Barinaga 1990). In 2004, through the Global Amphibian Assessment (GAA), the International Union for the Conservation of Nature (IUCN) tried to evaluate causes of decline and threat levels of almost every amphibian species. Results from these studies showed that 32.5% of all amphibian species are threatened with extinction and that 43.3% are declining (Stuart et al. 2004, 2008). Many factors seem to cause decline in amphibian diversity that may be associated with high frequencies of body anomalies and malformations (Pounds et al. 1997), and therefore many authors tried to identify their potential causes (Blaustein et al. 1997; Kiesecker 2002; Johnson and Chase 2004; Piha et al. 2006; Ballengée and Sessions 2009). Body abnormalities in amphibians have been documented in the literature for at least 300 y (e.g., Vallisneri 1706); however, much of the information on amphibian abnormalities is anecdotal, consisting of descriptions of malformation typically based on small sample sizes (see synoptic Table 1 in Henle et al. 2012). Information on the prevalence of morphological malformations at the population level is available for many frog species, but only occasionally for salamanders (Wheeler et al. 2002; Ferrer and López 2003; Miller and Miller 2005; Henle et al. 2012; Mačát et al. 2015). Information on the prevalence of one or more abnormalities have important implications at the ecology, biology, and health level of any population. External body malformations in amphibians can be attributed to both anthropogenic and natural factors. Causes for these abnormalities include parasites and pathogens, UV radiation, regeneration following trauma, high levels of anthropogenic pollution, intraspecific or interspecific relations, or synergistic interactions among some of these factors (Blaustein et al. 1997; Reaser and Johnson 1997; Gillilland and Muzzall 2002; Williams et al. 2008; Lannoo 2009).

Using a large dataset, consisting of more than 500 individuals from the same population, we analyzed the occurrence of malformations in the Northern Spectacled Salamander (*Salamandrina perspicillata*). We tested if sexes were differently affected by malformations and if some anomalies were more prevalent than others.

Finally, we tried to determine which, if any, of the main causes of malformations in amphibians are affecting salamanders in the study population.

Materials and Methods

Salamandrina perspicillata is endemic to Central and Northern Italy (Romano et al. 2009a) and is widespread along the Apennine Mountains, mainly in shady and damp areas but also in Mediterranean forests. The adults are terrestrial, but females enter water, typically slow running streams, to lay eggs (Lanza 1983). We sampled salamanders during 10 sampling days (October 2013 - May 2014) on the forest floor in one hectare of the Montedimezzo Regional Forest (41°45'N, 14°13'E; Molise region, Central Italy; about 900 m a.s.l.), a Supra-Mediterranean mixed deciduous forest dominated by Beech (*Fagus sylvatica*) and Turkish Oak (*Quercus cerris*). *Salamandrina perspicillata* at this site breeds in a stream and is the only urodele species observed at the study site. For each salamander, we determined sex using external cloacal morphology (Romano et al. 2009b) and we measured the snout-vent length (SVL) from the tip of the snout to the posterior margin of the cloaca. We calculated the operational sex ratio in accordance with Wilson and Hardy (2002) as the proportion of mature males: males/(males+females). Departures from equality were assessed by binomial test. To avoid repeated sampling of the same individuals, we photographed the belly of each salamander (Vanni et al. 1997) and used the software APHIS (Moya et al. 2015) for individual recognition based on the ventral pigment pattern. We carefully examined the dorsal, ventral, and lateral sides of digital images of each individual to detect body malformations.

Following the description of abnormality categories designated by Lannoo (2009) and by Rothschild et al. (2012), we recorded brachydactyly (short toe), ectrodactyly (missing toe), polydactyly (extra digit), and bifid tail. Before performing statistical comparison among groups, we used Levene's test for the homogeneity of variances of the compared groups and, based on these results, we applied parametric or nonparametric tests. We performed all statistical analyses using the package PAST (Hammer et al. 2001). We used a Student's *t*-test to test if individuals with limb abnormalities differed in size from healthy individuals. We also analysed intra-sexual size differences between malformed and healthy salamanders, differential prevalence of malformed individuals between the sexes, and, differential prevalence on limbs among the three observed deformities. We used Chi-square with fourfold table to test if malformations were related to sex. We used the Kruskal-Wallis (K-W) test and post hoc tests with Bonferroni correction to test if there was a significant difference in the prevalence of malformations (brachydactyly, ectrodactyly, and polydactyly). We applied these tests on the two sexes considering them both pooled and separated. We counted malformations considering each limb: if a salamander showed two malformed limbs, we recorded two abnormalities, one for each affected limb of the individual. We used $\alpha = 0.05$ for all tests.

Results

We sampled 508 adult *Salamandrina perspicillata*, 321 males and 187 females. The sex ratio (0.63, M/TOT) was significantly male-biased ($r = 134$, $P < 0.001$). We identified physical malformities in 42 individuals (8.3%) including 19 males (3.7%) and 23 females (4.5%). Among the malformed individuals, 63.4% showed brachydactyly, 61% ectrodactyly, 17.1% polydactyly (Fig. 1), and 0.2% bifid tail (Fig. 2). Because each individual could have more than one malformation (61.9% showed multiple malformations), we also examined whether multiple abnormalities affected one sex more by than the other.

Males (n = 321, mean SVL + SD = 37.6 + 8.82 mm, range 30–44 mm) and females (n = 183, mean SVL = 42.6 + 5.83 mm, range 32.8–49 mm) differed significantly in their size ($t = -24.46$; df = 503, $P < 0.001$). Consequently, the statistical tests, which also included the size, were performed on the two sexes separately. Within each sex, SVL did not differ between individuals with and without limb abnormalities (males: $t = 1.467$, df = 320, $P = 0.146$; females: $t = 0.215$, df = 186, $P = 0.830$). The percentage of females (12.3%) with malformations was significantly higher than that of males (5.9%; $X^2 = 5.44$, df = 1, $P < 0.05$). Morphological abnormalities differed significantly in their prevalence for all animals (H = 20.09, df = 40, $P < 0.001$), for males only ($H = 7.05$, df = 18, $P < 0.05$), and for females only ($H = 13.12$, df = 21, $P < 0.001$). Polydactyly was the only limb malformation showing significant lower prevalence compared to the other two types of abnormalities ($P < 0.001$ for all comparisons, against brachydactyly and ectrodactyly).

We recorded tail abnormalities on one salamander (2.4% of all malformed individuals): a female (SVL = 44.07 mm, TL = 89.58 mm) with a bifid tail (Fig. 2). The secondary tail was 13.44 mm long and branched from the right side of the primary tail (45.51 mm long) 12.1 mm posterior to the cloaca. The secondary tail was active and the individual was able to move the two tails at the same time in different directions. Both the primary and the secondary tail showed the same red

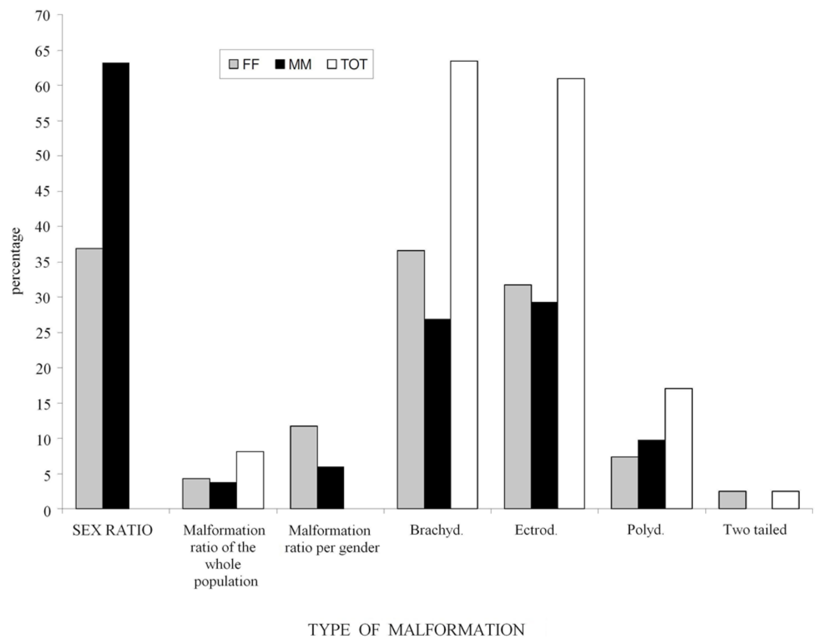

Figure 1. Histogram showing the frequency of the abnormalities of females (grey bars), males (black bars) and sexes pooled (white bars) of the Northern Spectacled Salamander (*Salamandrina perspicillata*) found during the study. Brachyd. = brachidactily, Ectrod.= ectrodactyly, Polyd. = polydactily, FF = females, MM = males, TOT = total.

ventral and dark dorsal pigmentation. The primary tail was shaped normally, whereas, the secondary tail was stumpy and blunt at the posterior end (Fig. 2).

Discussion

Although body malformations in amphibians may involve different body parts, the most common are those affecting eyes and limbs, and superficial abnormalities, such as incomplete tail resorption or skin trauma (Reeves et al. 2008; Hassine et al. 2011). Available information on body abnormalities in *Salamandrina* mainly concern chromatic anomalies, such as erythrism in a completely red individual, also red on dorsal side (Camerano 1884), partial albinism (Ramorino 1863; Lanza 1946), albinism (S. Tripepi in Angelini et al. 2007), xantism (Schreiber 1912), and polychrome dorsal spots (Lanza and Canestrelli 2002). Other morphological malformations in *Salamandrina* concern a case of polypody (Ramorino 1863) and a case of hemimely (Angelini 2000). Bifurcated tails have been reported for other species of urodeles but is a rare type of anomaly (Lynn 1950; Henle et al. 2012; Martínez-Silvestre et al. 2014). Bifurcated tail is usually explained as hyper-regeneration after mechanical injury caused by predators (Dawson 1932; Lynn 1950), such as tail pinching or partial amputation (Tornier 1900).

The results of our study offer information on a large dataset of salamanders belonging to a single population and, therefore, the frequency of the malformations here reported are not biased by a small sample size. The percentage of malformed individuals we found (> 8%) exceeds that expected in healthy populations of amphibians, which should be < 5% (Meyer-Rochow and Asahima 1988; Ouellet 2000; Stocum 2000; Johnson et al. 2001; Blaustein and Johnson 2003). Even if these thresholds are mainly based on studies on anurans, the available information on urodeles are concordant (Hiler et al. 2005; D'Amen et al. 2007; Martínez-Silvestre et al. 2014). In central Italy, the causes of differences in the prevalence of malformed individuals of two species of newts (*Triturus carnifex* and *Lissotriton vulgaris*) in three localities were considered proportional to either different levels of agriculture in those areas or alpha radiations from Radon decay in a volcanic area (D'Amen et al. 2007).

Furthermore, our findings are unexpected because most malformed individuals come from lentic water habitats; whereas, amphibians inhabiting lotic environments are less affected by malformities (see Sparling et al. 2010). During the past few decades, the number of populations of species of amphibian with abnormally high percentages of malformed individuals has increased, and long term studies indicate an increasing trend of frequency (see Sparling et al. 2010 for a review). Genetic, environmental, nutritional, and parasitological factors, often acting synergistically, may be important causes of malformations in amphibians.

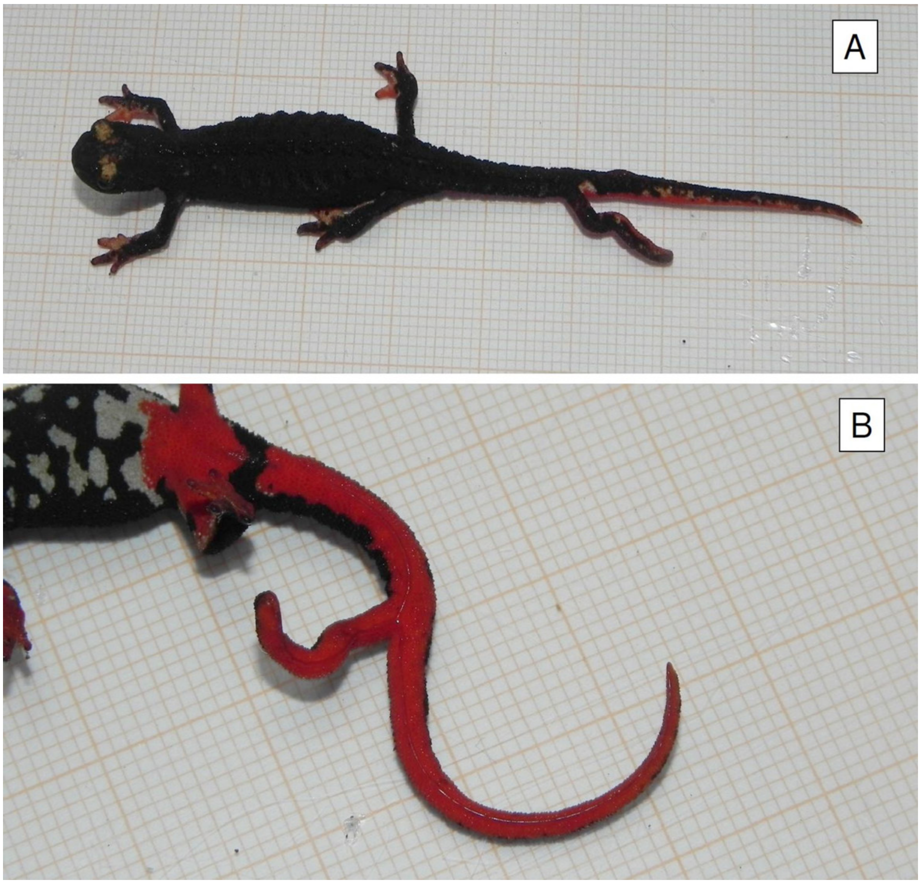

Figure 2. A) Dorsal view of the female Northern Spectacled Salamander (*Salamandrina perspicillata*) exhibiting a bifid tail. B) Ventral detail of the tail. (Photographed by Antonio Romano).

They may be pooled in five main categories: hyper-regeneration after predator attempts, exposition to high UV-B radiation, chemical pollution, environmental degradation, and parasite infection (Lannoo 2009; Sparling et al. 2010; Mačát et al. 2015 and references therein). The identification of the exact cause or causes is often difficult. Brachydactyly and ectrodactyly are generally considered skeletal injuries; whereas, polydactyly is considered a consequence of skeletal malformations (Reeves et al. 2008; Hassine et al. 2011). The population we studied experiences a low level of UV radiation (because of the presence of a well-structured tree canopy cover), and probably a low number of potential predators that could injury salamanders (cfr. Angelini et al. 2007). To explain the high frequency of salamanders with body anomalies in this population, we suggest three potential causes, which are not mutually exclusive: trematode infections, physical injuries related to flood regime, and trampling by ungulates. The latter acts on both sexes equally, the others may explain the differences between sexes. Trematode infection potentially affect populations of many species of salamanders (e.g. Wyderko et al. 2015), can cause limb malformations (Johnson et al 1999; Sparling et al. 2010), and increase mortality rates (Kiesecker 2002; Johnson and Hartson 2009). The life cycle of many trematodes requires an intermediate host with cercariae and metacercariae developing in a snail. In aquatic habitats, cercariae may escape from the snail and encyst as metacercarie in the skin of larval or adult amphibians (Mitchell 2008). Adult amphibians generally become infected from ingesting snails or ingesting their sloughed epithelium with encysted metacercariae (Mitchell 2008). Sexes may have different encounter rates with parasites (De Lisle and Rowe 2015) because of different behaviors. In explaining the different infection rate between males and females of *Salamandrina*, sexual eco-ethological differences could be involved. In this species, adult males are entirely terrestrial; whereas, females enter water for prolonged periods to spawn (Lanza 1983). Therefore, females could be more exposed to trematode infection. Although this species is considered a fully terrestrial predator (Lanza 1983; Salvidio et al. 2012), aquatic prey have been found in the stomach of a few salamanders (Ramorino 1863) and, considering that gastropods are preyed on by *Salamandrina* (Salvidio et al., 2012), aquatic snails that are intermediate host of trematodes could be eaten by females. Furthermore, only females are exposed to skin infection during their aquatic phase, when cercariae may encyst in the skin of an amphibian (Mitchell 2008).

Physical injuries are a second possible cause of the observed body anomalies. The trampling of ungulates, which acts equally on both sexes, may cause severe skeletal injuries that could lead to brachydactyly and ectrodactyly (Reeves et al. 2008; Hassine et al. 2011). Indeed, the Montedimezzo forest is a nature reserve with a very little anthropogenic influence but a high disturbance level because of the presence of Wild Boars (*Sus scrofa*) that affect plant forest turnover (Sabatini et al. 2014). Aquatic phase of females may be responsible also for the different ratio of malformation observed between sexes. Mediterranean streams are subject to unpredictable and frequent floods during early spring. Drifting of reproductive amphibian females may reduce longevity in natural populations (e.g., Tucker et al. 2001; Cogalniceanu and Miaud 2003; Wheeler and Welsh 2008). *Salamandrina* have a limited swimming ability, and can be easily carried away by streams and flooding. Populations of *Salamandrina* breeding in running water suffer higher mortality than populations breeding in lentic water (Angelini et al. 2008). Violent flooding events may be another factor that could cause physical injuries to females leading to hyper-regeneration. Finally, *Salamandrina* is recognized to be the sole extant representative of an ancient lineage of the family Salamandridae (e.g., Titus and Larson 1995; Steinfartz et al. 2006) and any comparison with other amphibians should be regarded with caution. Further studies on other *Salamandrina* populations are needed to understand if this percentage of body abnormalities is a characteristic of this species or it may be considered as an alarm signal about the health of the studied population.

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