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Population density, sex ratio and body size in a population of *Salamandra atra atra* on the Dolomites

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Abstract. *Salamandra atra atra* is the most widespread subspecies of the Alpine Salamander, both in Italy and in the other parts of the species distribution range. However, in particular for Italian populations, its ecology and demographic parameters are poorly known. We studied biometry (length, mass, body condition index) and demography (population density, sex ratio, proportion of gravid females) of this fully terrestrial salamander in the "Paneveggio-Pale di San Martino" Natural Park in the Dolomites. We used removal methods to estimate abundance on a surface of about 1000 m^2. Density estimate of adults was 472 salamanders/ha, which falls within the density estimates that are available for this taxon. Sexes did not differ significantly in size and body mass. Body sizes of adults included the maximum sized salamander recorded in Italy. There was a high rate of gravid females (50%), which were comparable in size with non-gravid females. Males and non-gravid females did not show significant differences in their body condition index.

Keywords. Amphibian, body condition, population density, salamanders.

Salamandra atra Laurenti, 1768 is a terrestrial and viviparous salamander. The species occurs in the Central and Eastern Alps and in the Dinaric Alps where some isolated populations occur (Sillero et al., 2014). Four subspecies are generally recognised: the nominal *S. a. atra*, *S. a. prenjensis* and the Italian endemics *S. a. aurorae* and *S. a. pasubiensis*. Italian populations of the nominate subspecies are generally found from 1200 to 2200 m a.s.l.; in the Dolomites (Italian part of the southern limestone Alps), the species occurs from 650 m up to 2500 m (Bonato et al., 2007). The habitat consists of mixed coniferous and deciduous forests, alpine meadows and rocky tundra-like, mainly on limestone substrates. Although *S. a. atra* is the most widespread subspecies (also in Italy), ecological, morphometric and demographic information are scarce and, for Italian populations, almost completely absent (see table 3 in Bonato et al., 2007). Indeed, exhaustive information is available only for a population from the easternmost part of the Italian Alps (Luiselli et al., 2001). Here we reported the results of a study on a population of *S. a. atra* on the Dolomites. We collected and analysed data on density, body size, weight, sex ratio and proportion of gravid females and we compared our results with those available in literature.

The study area is located in the "Paneveggio-Pale di San Martino" Natural Park (Trentino Alto Adige region), at about 1850 m a.s.l., near the locality Malga Venegiotta (Tonadico; 46°18'48"N, 11°48'53"E). The study area consists of coniferous woodland dominated by the European larch (*Larix decidua*) and Norway spruce (*Picea abies*),

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alternated to open habitats (pastures, other grasslands and rocky areas). Salamanders were searched by eye during their surface activity on the ground by two researchers, during both day and night hours on 1st September 2017 at temperatures of 3-18 °C, during optimal weather condition (slightly rainy or just after rain). Two sites were sampled. The first (AA) was a polygonal area of 890 m^2, where salamanders were searched for one hour during three sampling sessions (first session: 6:00-7:00; second: 12:00-13:00; third session: 22:30-23:30). The site AA is an open habitat on a stony slope with south-western orientation, dominated by *Erica carnea* and herbaceous vegetation, on the boundary of a small stream, near a trekking path. The second site (BB) was a portion (about 400 m long, 2-3 m width) of this trekking path, which have to be travelled to reach the area AA. In the site BB salamanders were sampled only once (21:30-22:30). Population size was estimated in the site AA, using the software CAPTURE (Otis et al., 1978; White et al., 1982) with a temporary removal experiment (White et al., 1982), with the three removal samples within 18 hours. The removal method theory assumes that population is closed and that the sampling effort is constant among the sampling sessions (White et al., 1982). We met these assumptions by sampling salamanders over a very short time period (18h) for a standardised time (1 hour). We used the generalized removal model M_{bh} that allows for variation in capture probabilities (*b* allows for capture probabilities to vary by behavioural response or "capture history"; *h* is the individual heterogeneity effects and allows for capture probabilities to differ between individuals). Salamanders collected in the first and second removal sample were stored at 4 °C in a refrigerator in individual plastic tubes for a maximum of 20 hours near the study area (about 6 km). For the evaluation of the sex ratio, proportion of gravid females, body mass and size, we pooled the salamanders sampled in the site AA and those in the site BB. Salamanders were considered as "adults" when they were longer than 90 mm in total length (Klewen, 1986; Luiselli et al., 2001).

Salamander sex was assessed by direct observation of the cloacal region, which is swollen in the adult males and flat in females (Klewen, 1988). Females were considered "gravid females" when their pregnancy was obvious, that is the posterior part of their trunk was distinctly swollen laterally because they were at a late stage of gestation (Klewen, 1988; Bonato et al., 2017) and when, for females whose trunk was swollen but not so evident, our gentle palpation of their belly confirmed their pregnancy (Andreone et al., 1996; 1999; Luiselli et al., 2001). The "non-gravid females" included presumably both actually non-gravid ones and females with embryos in early stages of development. Throughout the text, "females" include both gravid and non-gravid females.

In accordance with Wilson and Hardy (2002), sex ratio was expressed as the proportion of males [males/(males+females)] and deviations from evenness were assessed by two-tailed binomial tests. Salamanders were photographed perpendicularly to the dorsal surface with a digital camera, near a calliper to set the scale. Digital photographs of salamanders were imported into the ImageJ® software program (version 1.44; National Institute of Health, available from http://rsb.info.nih.gov/ij/) to measure their total length, after scale setting of each picture (TOTL: distance from the tip of the snout to the tip of the tail; precision 0.1 mm) while body mass was measured using a digital scale (precision 0.01 g). To quantify the magnitude of sexual size dimorphism, we used the sexual dimorphism index, SDI = [(mean TOTL of the larger sex/mean TOTL of the smaller sex) − 1], arbitrarily defined as positive when females are larger and negative when males are larger (Lovich and Gibbons, 1992). In addition, a maximum size dimorphism index (SDImax) based on the mean TOTL of the largest 20% of the sample of each sex was also calculated (du Toit et al, 2003; Romano et al., 2009).

TOTL and mass were combined to calculate the body condition index as Scaled Mass Index (SMI) (Peig and Green, 2009). The SMI was calculated only for males and non-gravid females, while the proportion of gravid females on the total number of females may be used as a proxy of the fertility parameter (Luiselli et al., 2001). Since our biometric data did not match parametric assumptions, body size, body mass and SMI of males, females and gravid females were compared using the non parametric Mann-Whitney-U test and Kruskal-Wallis Anova.

All salamanders captured were adults (TOTL > 90 mm, see Klewen, 1986; Tab. 1). In site AA we captured 24 individuals (6, 9 and 9 individuals in the first, second and third removal session respectively). The estimated abundance of salamanders was 42 ± 7 (estimate ± s.e.; 95% C.I. = 33-62). Therefore salamander's density, computed as the estimated abundance in 890 m^2 (site AA) extrapolated to the hectare, resulted 472 individuals/ha (95% C.I = 310-633, calculated following the Delta method as reported in Cooch and White, 2018). In site BB, during a single sampling session (slightly rain, night), were found 27 salamanders.

On the whole, pooling salamanders from sites AA and BB, we measured, weighed and sexed 51 salamanders (31 males, 20 females). Our results showed a sex ratio of 0.61 which did no differ significantly from 0.5 (two tailed binomial test, P = 0.119). Body size and body mass of males, non-gravid females and gravid females

are shown in Tab. 1. Although males are generally longer than females, sexes did not differed significantly in their mean size (Mann-Whitney U test: N = 51; U = 222; P = 0.09) and body mass (comparison between males and non-gravid females: N = 41; U = 129.5; P = 0.44). Gravid and non-gravid females had comparable sizes (N = 20; U = 42; P = 0.58). Kruskal-Wallis Anova, followed by Mann-Whitney pairwise comparisons with Bonferroni correction for multiple comparisons, showed that gravid females differed significantly in body mass both from males and non-gravid females (Kruskal-Wallis; H = 6.69; P = 0.03; P < 0.05 in both statistically significant comparisons). The sample of salamanders we studied showed a slightly negative SDI = -0.05. Conversely, the SDImax showed a positive value (+0.11), because although males are generally larger than females the largest individuals in our sample were females. Males and non-gravid females did not show significant differences in their body condition index (SMI) (Mann-Whitney U test; N = 41; U = 124, P = 0.36).

Information on ecology, morphometry and demographic parameters of Italian populations of Alpine Salamanders (*S. a. atra*) are scarce (cf. Bonato et al., 2007). Most of the results we obtained corroborate available data from other populations of Alpine salamanders. Population density of *S. a. atra* seems to be highly variable among different sites, spanning from more than 3000 individuals/ha (Helfer et al., 2012) to 120 ind./ha (Klewen, 1986), with intermediate values (1383 ind./ha: Helfer et al., 2012; 2380 ind./ha: Klewen, 1986, 1988). Density of the subspecies *S. a. aurorae* ranged from 475 to 40 ind./ha (Bonato and Fracasso, 2003; Romano et al., 2018a). Spatial distribution of *Salamandra atra* may be significantly aggregated also within small areas (about 1300 m^2; Bonato and Fracasso, 2003). Consequently, extrapolation of population density from very small areas to hectares should be regarded with caution because could greatly under- or over-estimate the actual densities at larger scale. The highest density of *S. atra* (3056 ind./ha, Helfer et al., 2012) was extrapolated on an hectare from a small study area (625 m^2) while lower values were extrapolated by larger areas (3000 m^2) and, in our opinion, they are more reliable and are closer to the actual density of this salamander. In our population the observed density of about 470 ind./ha was comparable to those in areas of maximum density of the subspecies *S. a. aurorae* in the Venetian Prealps (Bonato and Fracasso, 2003). In our three removal sessions we did not observe decline in number of captures which may indicates a poor capture success (e.g., Rodda, 2012).

In the third sampling session (slightly rainy night) we found 9 salamanders in the site AA and 27 in the site BB where no animals were detected in the first two sessions. This corroborates available information about the Alpine Salamander detectability which may vary with site characteristics (like deep cracks within rocks) and with survey conditions (weather) (e.g., Bonato et al., 2007).

Body size data of the study population generally are in agreement with those available in the literature. Maximum size (TOTL) and mass recorded in Italian populations of *S. a. atra* were 140 mm and 11.8 g, 143 mm and 14.2 g for males and females respectively (Luiselli et al., 2001; Bonato et al., 2007). For the whole subspecies the maximum records are still 144 mm and 12 g, 151 mm and 15 g for males and females, respectively (Bonato et al., 2007) and our data are congruent (see Table 1). However, two males in our study population (140.3 mm and 143.4 mm, Tab. 1) exceeded the maximum size recorded in other Italian populations. As a rule, there is no significant sexual size dimorphism in Alpine salamanders (Bonato et al., 2007), as it was the case for our study population.

The sex ratio and the proportion of gravid females (at least 50% of the females were gravid) corroborated the information reported in previous studies form other populations (Luiselli et al., 2001; Bonato et al., 2007). Here we assumed a negligible variation in detectability between males and females. However, available information on different survey methods on *S. a. aurorae* showed that they provide different level of efficiency in detecting males and females (Lefosse et al., 2016). The method we used (searching active salamanders on the ground during slightly rain) could provide a similar efficiency in detect-

Table 1. Body size and body mass of *Salamandra atra atra* from the study site in the "Paneveggio-Pale di San Martino" Natural Park (Trentino Alto Adige region, Northern Italy). "Females" are the gravid and non-gravid females pooled together.

	N	Total length (mm)		Body mass (g)	
		mean ± s.d	range	mean ± s.d	range
Males	31	122.6 ± 12.8	94.4-143.4	8.1 ± 1.9	4.2-12.0
Females	20	116.3 ± 13.1	95.7-145.4	8.6 ± 2.3	4.2-12.4
Non-gravid females	10	114.5 ± 15.2	95.7-145.4	7.5 ± 2.3	4.2-11.8
Gravid females	10	118.0 ± 11.2	103.0-139.6	9.7 ± 1.7	6.4-12.4

ing both sexes (Lefosse et al., 2016). However *S. a. aurorae* shows some ecological and behavioural characteristics that are different from those of the nominate subspecies (Bonato et al., 2007; Lefosse et al., 2016). As general rule, in salamanders males show higher capture rates than females (Lefosse et al., 2016; Romano et al., 2018b) but although the sex ratio obtained by counts may overestimate one sex (generally the males) it can be congruent with the actual demographic trait of the population (Romano et al., 2018b). Further studies based on CMR are desirable to understand if the observed sex ratio reflects an actual ecological trait of the population or is the outcome of different capture probabilities.

In our population, gravid and non-gravid adult females had comparable sizes (Table 1). This finding is not in agreement with the data of other populations where gravid females were significantly larger (cf. Luiselli et al., 2001) but this discrepancy could be due to our small sample size (20 females).

Body condition is a parameter in many ecological and monitoring studies (e.g., Welsh et al., 2008; see also Tab.1 in Milenkaya et al., 2013). The use of condition indices in conservation needs to control for the possible confounding effects of sex, because body condition may differ between males and females (see Milenkaya et al., 2013). If it differs between sexes, the individuals cannot be pooled for spatial (different populations) or temporal (the same population in different times) comparisons because results may be affected by the sex ratio. Here we showed that in *S. atra* the sexes (males and non-gravid females) did not differ in body condition index and, therefore, they may be pooled in population studies that take into account this parameter.

This contribution provides additional information on little studied aspects in the Alpine salamanders in Italy, such as population density, body sizes, proportion of gravid females and body condition index. Furthermore we confirms that this area is suitable to more in-depth researches, which are in planning to study distribution, abundance and ecology of *S. a. atra* in the "Paneveggio-Pale di San Martino" Natural Park.

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