

The Variation in Black and Yellow Spot Color Patterns Among Three Populations of the Near Eastern Fire Salamander (*Salamandra infraimmaculata*)

Gad Degani ^{1*}, Gad Ish Am ², Amit Biran Ish Am ², Amir Marshansky ³, Sivan Margalit ¹, Eitan Nissim ⁴, Hava Goldstein ⁴, Niva Ahkked ¹

¹ MIGAL – Galilee Research Institute, Kiryat Shmona, Israel; Faculty of Science and Technology, Tel-Hai Academic College, Kiryat Shmona, Israel

² Kibbutz Sasa, Upper Galilee, 1387000 Israel.

³ Kibbutz Yehiam, 2512500 Israel.

⁴ Israel Nature and Parks Authority, Israel.

***Corresponding Author:** Gad Degani, MIGAL Galilee Research Institute, Kiryat Shmona, Israel; Faculty of Science and Technology, Tel-Hai Academic College, Kiryat Shmona, Israel.

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Abstract

Proper use of medicine or taking medicine in correct order is essential to cure any disease. According to the WHO, lack of adherence to treatment regimens leads to major problems among patients, mostly with chronic illnesses. "Right administration" depends on at least 5 right factors right patient, right drug, right time, right dose and right route

Keywords: salamandra infraimmaculata; color pattern; population; habitat; spot; yellow

Introduction

The genus *Salamandra* Garsaut, 1764, belonging to the terrestrial Urodeles, is widely distributed in Europe and reaches, at its southern border, North Africa and the Middle East [1] (Figure 1). The systematics of this genus has undergone many changes, from its division into subspecies to its generally

agreed upon division into six species [2-4]. Due to the large variation in salamander types, classification into species and subspecies is complicated and ambiguous. Some of the six species of the genus *Salamandra* are defined as subspecies in their wide distribution in Europe, North Africa and Asia, including Asia Minor and Israel [4] (Figure 1).

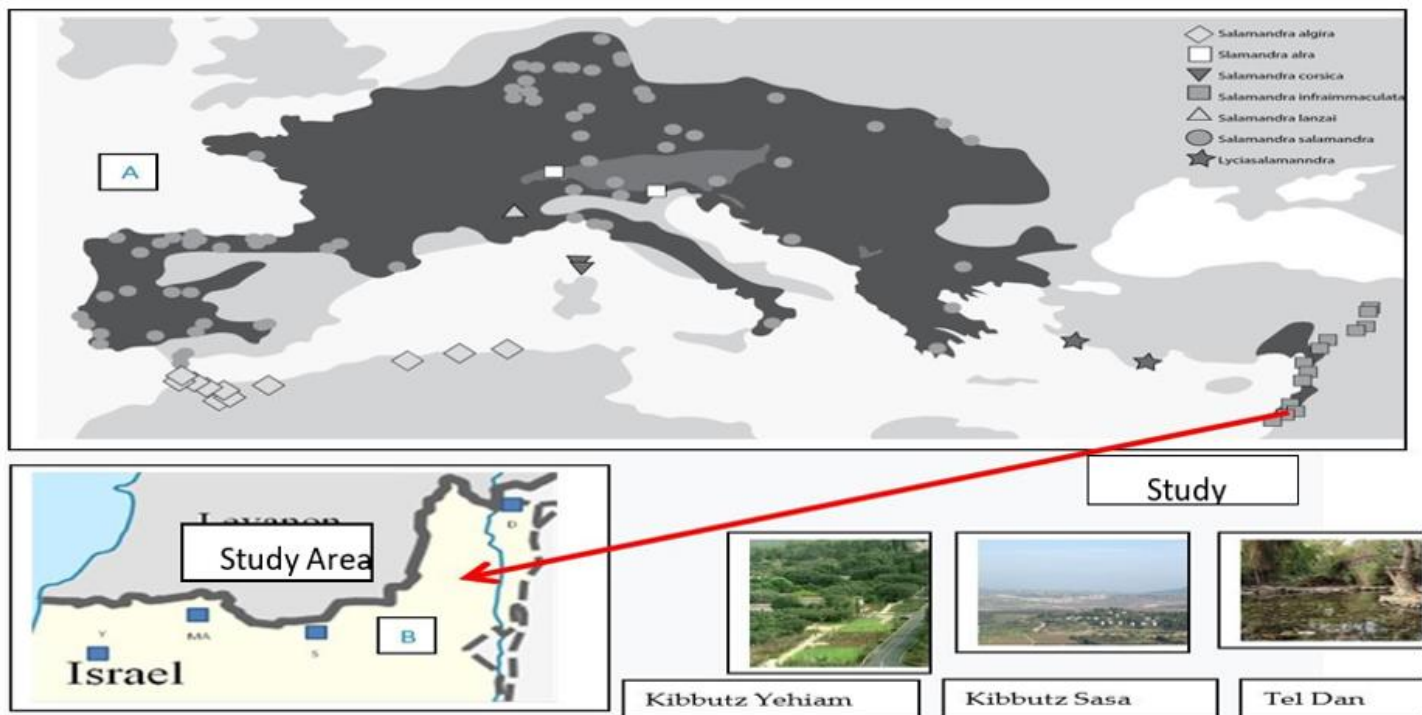


Figure 1: Distribution of the genus *Salamandra* in Europe [2,5]. (A) *Salamandra inframaculata* distribution and (B) study area [6].

Locations of the studied populations (study sites) appear on this map: Kibbutz Sasa ($33^{\circ}1'37''\text{N}$ $35^{\circ}23'40''\text{E}$, 910 m above sea level [M.A.S.L.]) [7], Kibbutz Yehiam ($32^{\circ}59'39''\text{N}$ $53^{\circ}13'19''\text{E}$, 634 M.A.S.L.), Tel Dan ($33^{\circ}14'25''\text{N}$ $35^{\circ}39'11''\text{E}$, 190 M.A.S.L.) [8].

This division and the large variation in colored spot patterns on the backs of salamanders among populations sometimes make it difficult to determine the species or subspecies with any certainty [1]. Steinfartz et al. [2] suggested classifying the species in Europe, North Africa and The Levant into six groups based on their mitochondrial DNA: *Salamandra salamandra*, *Salamandra inframaculata*, *Salamandra corsica*, *Salamandra atra*, *Salamandra lanzai* and *Salamandra algira*. Accordingly, the fire salamander (*S. inframaculata*) is located in Israel and *S. algira* in North Africa [9,10]. The coloration patterns were then associated with habitat type and geographical area of distribution [11]. For the comparison, salamanders in Israel were taken from the Mount Carmel area. Those researchers gave a first description of the karyotype of the fire salamander in Israel, finding 12 pairs of chromosomes [11].

In Israel, where the salamanders are located on the southern diaspora border, there are isolated populations which have survived in the north of the country. Due to the different environmental conditions in their habitats, a comparison of these populations could be interesting [1,12,13], in terms of morphological [14] and physiological [15-17] variations, larval growth [18], and genetic variations under different environmental conditions [5,19-21].

S. inframaculata shows a fragmented distribution in various types of habitats, including mountain ranges that are 130 to more than 1000 m above sea level [1,22]. Due to polymorphic coloration and a diversity of reproductive modes, it is very difficult to separate the species according to these variables [5]. Genetic studies have focused mainly on tadpoles in the various populations. Samples of salamanders after metamorphosis usually consist of relatively small numbers and determining the relationship between the morphological variance and molecular variability of several markers is problematic [5,22-24]. The color pattern of *S. salamandra* has been relatively

well-described in many articles (see for review [25]). The arrangement of the colored segments on their black back varies from two yellow lines on *S. terrestris* to variously shaped yellow spots on *S. salamandra*, and there is great variability among individual details, including combinations of these two models [5,25]. The differences between the color-pattern phenotypes in *S. salamandra* and *S. terrestris* are very large not only between species, but also among individuals. In Israel, where the species *S. inframaculata* lives under the most extreme conditions, and where the differences between habitats may affect the variability of the populations, morphological differences were studied in several areas based on a relatively small number of adult salamanders: the Tel Dan habitat, with constant conditions and water availability year round, the Upper Galilee, and the Western Galilee (Mount Meron, Kibbutz Sasa and Kibbutz Yehiam), with mountain habitats that are dry and warm in summer and cold and rainy in winter [1,3,14]. The body size of the salamanders from the Tel Dan population was significantly smaller than that of salamanders from the other areas. These differences were supported by studies of the genetic differences

between the Tel Dan population and salamanders in other parts of Israel living under more extreme ecological conditions [1,21]. In studies on the pattern of yellow spots on the back of salamanders between different regions of Israel (Tel Dan, Upper Galilee, Western Galilee and Mount Carmel), there were no clear differences between the regions [3]. We hypothesized that the adaptation of salamanders to extreme environmental conditions will be reflected in the Kibbutz Sasa (Sasa) and Kibbutz Yehiam (Yehiam) populations by morphological and physiological changes compared to salamanders in the Tel Dan (Dan) population, which live under less extreme conditions [1,21]. In the present study, we examined the potential differences among the spot patterns of the three different populations of *S. inframaculata*. We describe the different color-pattern phenotypes on the black backs of *S. inframaculata* in the various habitats: Dan (stable conditions throughout the year), Sasa near the Mount Meron region, and Yehiam in the Western Galilee. We further compare the color-pattern

phenotypes of *S. infraimmaculata* to those of other salamander species [5] (Figure 1).

Material and Methods

The study areas included Kibbutz Sasa and Kibbutz Yehiam, both semi-arid habitats in the region of one of the highest mountains in Israel, Mount Meron (Figure 1). The winter in this area is longer and rainier than in most of the rest of the country, with annual precipitation reaching 1000 mm.

The summer is comparatively dry, with no rain. The Tel Dan Nature Reserve is in the northeast of the country; it lies among hills and is bounded by mountains to the north, but is itself only 180 m above sea level. The area as a whole is characterized by numerous spring-fed streams with running water, and is the only such area in Israel.

Field observation, samples and photographs of salamanders for coloration pattern determination were from populations that have been intensively studied and for which all other parameters (body weight and length) have been well- described [1]. Of all of the salamanders that were photographed, only those that showed a clear difference between a black back and yellow spots were taken as samples for this study. Each salamander was individually inspected for its unique pattern of yellow spots on its head and back. Observations were conducted on rainy days when the salamanders are active on the surface in the three habitats and easy to find at night [7,26]. In the Dan population, 454 salamanders were photographed, of which 100 were sampled to measure the percentage of yellow and black coloring. In Sasa, 201 salamanders were photographed, and 62 sampled for color measurements. In Yehiam, 200 salamanders were photographed, of which 60 were sampled for color measurements. In all locations, about a third of the salamanders were photographed more than once. The number of spots on the head and back of each individual salamander was determined. The images were entered into the computer and the ratio of black to yellow color was calculated as the percentage of yellow color using the formula $[\text{Yellow}/(\text{Yellow} + \text{Black})] \times 100$. The photograph

of the salamander was copied into Word Object design software, giving it a white background. The image was then copied to the software program Cool PHP Tools Image Color Extract, where the percentage of yellow on the salamander's back was determined.

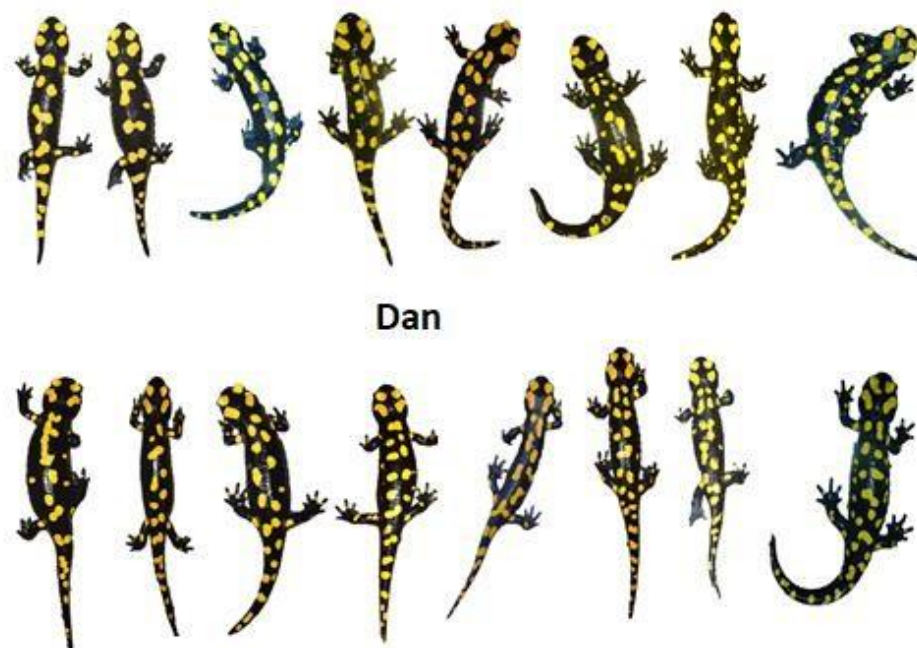
Statistical Analysis

All statistical processing was done using JAMOV 2.0.0 software. To check for differences in the number of yellow spots on the salamanders' back and in the black-to-yellow ratio among the various populations, we used several statistical tests. We used one-way analysis of variance (ANOVA) and post-hoc Student t-test for each pair of averages, without correction based on multiple tests.

ANOVA was used to examine the variation among all three populations in the number of yellow spots on the back and the black-to-yellow ratio. The t-test was used to compare between two populations. The number of salamanders with different color patterns in the various populations was estimated by Z-proportion analysis. The significance threshold was $p < 0.05$. Statistical processing was done with AMOVI software, t-test, Z-test and ANOVA.

Results

The color of the spots differed among individual salamanders in the same population, with various different shades of yellow. The patterns of the yellow spots on the backs of the salamanders in the different populations (Dan, Sasa and Yehiam) are shown in Figure 2: one row, two rows or scattered. The size and shape of the spots differed; some were round and others had different shapes. It was difficult to discern the differences in spot patterns between the different populations (Figure 2). A comparison of the patterns of yellow spots on the backs of the salamanders (one row, two rows and scattered) between populations (Dan, Sasa and Yehiam) is shown in Figure 3. Differences were not significant (t-test, Z-test and ANOVA, $p > 0.05$).



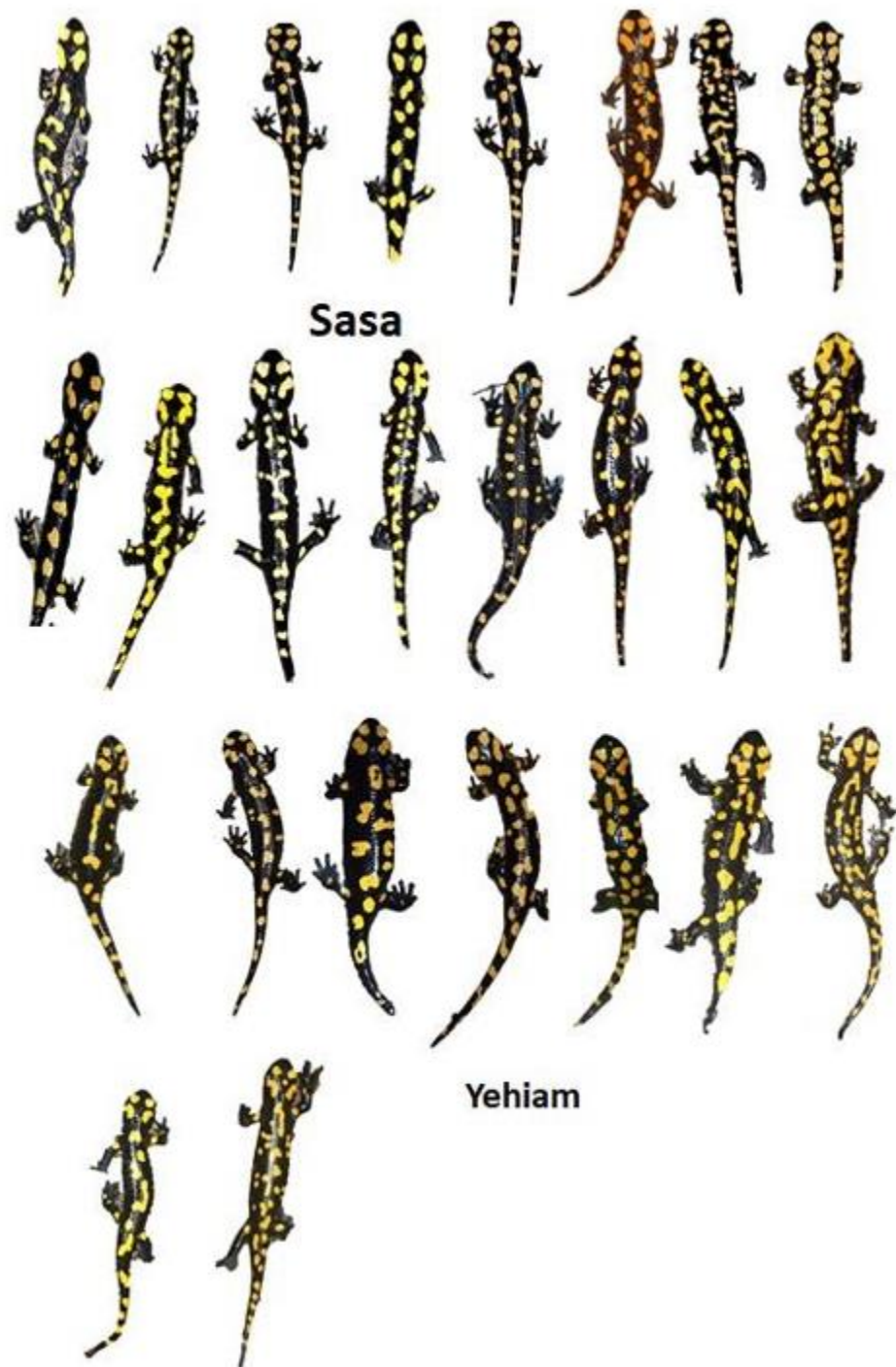


Figure 2: Different dispersion of spots on the back of the salamanders in the Dan, Sasa and Yehiam populations. Patterns appearing on the salamander body were: one row, two rows or scattered. The size and shape of the spots differed among individuals belonging to the various populations; some were round and others had different shapes.

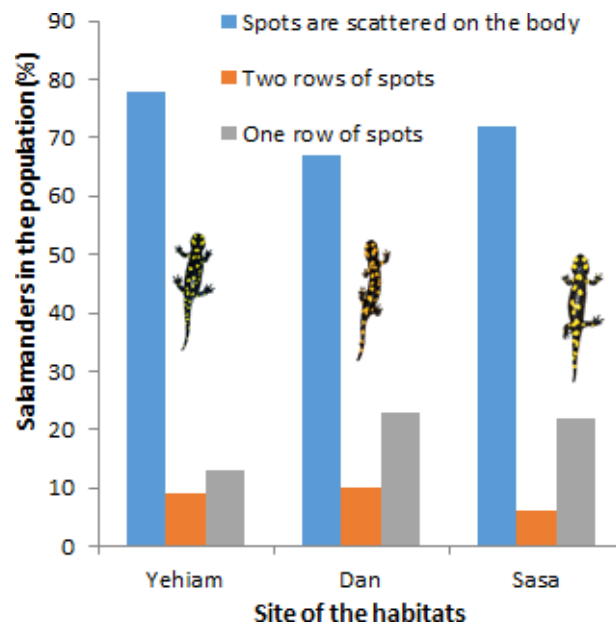


Figure 3: Comparison of the patterns of yellow spots on the backs of the salamanders (one row, two rows and scattered) between habitats (Dan, Sasa and Yehiam). Differences were not significant (Z-test, $p > 0.05$).

For two indices (proportion of yellow/black and number of spots on the head), the Dan population differed from the two other populations of salamanders (t-test, Z-test and ANOVA, $p < 0.05$). The ratio between yellow and black differed significantly between the Dan population and the other two populations, with the percentage of yellow on the back being significantly larger for the former (Figure 4) salamanders (t-test, Z-test and ANOVA, $p < 0.05$).

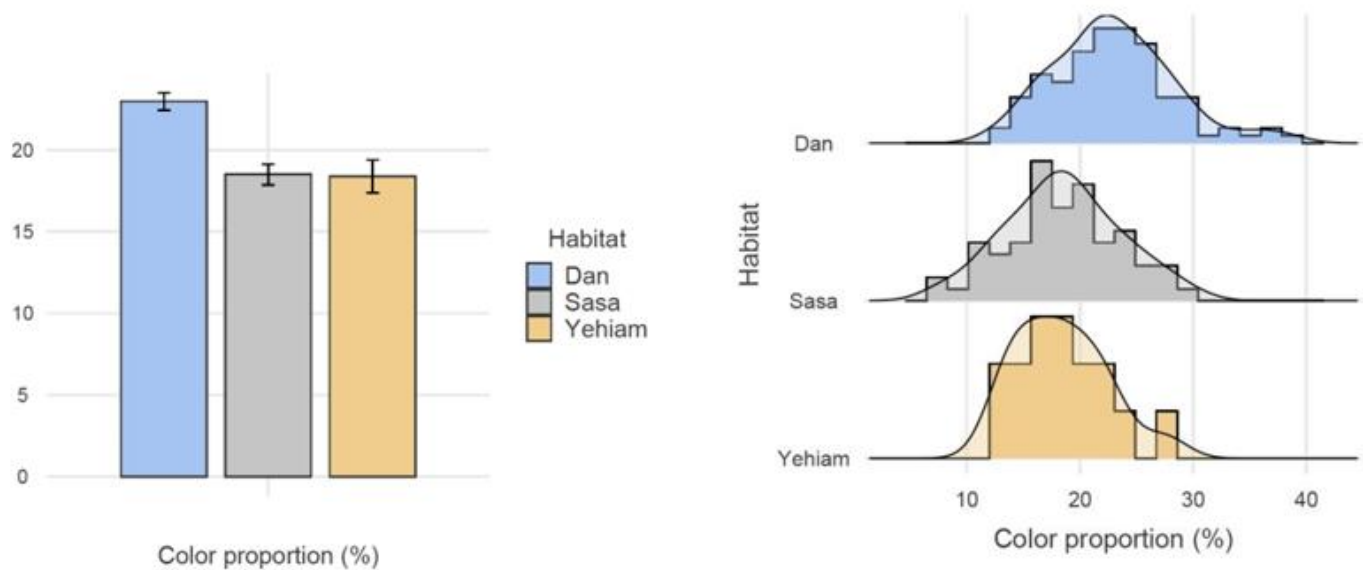


Figure 4: Comparison of percentage of yellow spots on the black back of salamanders in the different habitats (populations) using the JAMOV statistical program (ANOVA and t-test). No significant differences were found among the Yehiam and Sasa populations. Both populations differed from the Dan salamanders. The formula for calculating the percentage of yellow color was $[\text{Yellow area}/(\text{Yellow} + \text{Black areas})] \times 100$.

Various numbers of yellow spots were found on the head of the salamanders in the three populations, from 1 to 7 (Figure 5). In all populations, there were more salamanders with 4 spots than with any other number of spots. In the Dan population, there were significantly more salamanders with 1 to 3 spots than in Sasa or Yehiam. Here again, the spot pattern for the Dan population

differed significantly (ANOVA, $p < 0.05$) from those of the Sasa and Yehiam salamanders (Figure 5). No difference was found in the number of yellow spots on the head between Sasa and Yehiam salamanders (ANOVA, $p > 0.05$).

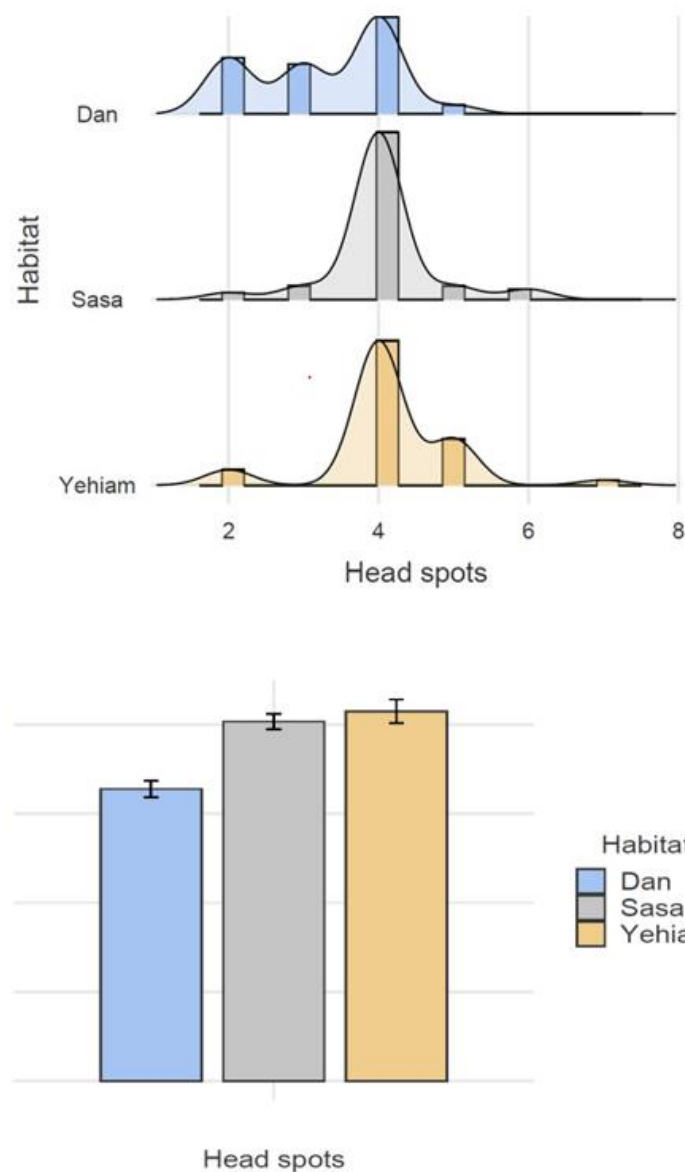


Figure 5: Number of spots on the heads of salamanders from the different populations (habitats - Dan, Sasa and Yehiam).

The color of the spots varied from light yellow (yellow with white hue) to yellow with a red hue in all populations studied (Figure 6).

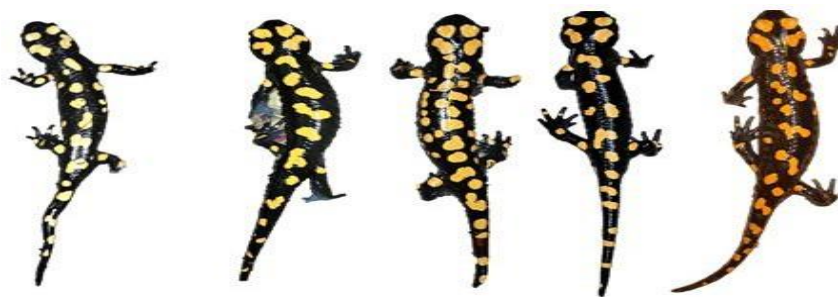


Figure 6: Different shades of spots on Sasa salamanders.

Discussion

Results of this study of spot patterns on *S. infraimmaculata* at the southern border of its distribution in three habitats revealed differences compared to the salamanders in Europe (Figure 7), in agreement with a relatively large

number of studies (e.g., [4-6]). The color pattern described for the various subspecies of *S. salamandra* (Figure 7C) appeared in the findings of this work, as well as the patterns seen in the water salamander *S. algira* [9,10] and *S. corsica* [5]. *S. salamandra* has the widest distribution among species

of the genus *Salamandra*. Most of the patterns of the dorsal spots appeared in both *S. salamandra* and *S. terrestris*. In the past, *S. infraimmaculata* was classified as *S. salamandra* mainly according to the spots on its back, hence the importance of the holocaust for these two species [1].

The contribution of this work is in the relatively large number of *S. infraimmaculata* individuals sampled at the southern border of their distribution, in different habitats that are relatively close together physically but have different environmental conditions: the Dan habitat, where conditions are constant and water is available year-round at constant temperatures [8], and Sasa and Yehiam, which are semi-arid habitats where it rains only a few months in the winter [1,7,26]. The hue of the spot colors found in this work appears in the genus *Salamandra*, but probably varies among species [4-6,28]. Differences were found in the arrangement of the spots between one population (Dan) and the other two (Sasa and Yehiam). The differences found in this study are supported by different biological aspects among populations of salamanders on the southern border of their distribution: morphological (body size) [1,14], physiology [15,16] and genetics [1,3,19], among others. Similar phenomena have been found in

salamanders of the same genus but different species (*S. algira*) at the southern border of their distribution in North Africa (Algeria) [29], in terms of both genetic variation among populations and spots on the back. The pattern of spots found in Israel on *S. infraimmaculata* in the present study has also been seen in *S. algira* in North Africa at the southern border of its distribution; however, there are also different models and colors [5,6,29]. Effects of ecological conditions on morphological variations have also been found in other species of amphibians, such as the green toad [30]. To the best of our knowledge, this study is the first to measure the relationship between the pattern on the back of *S. infraimmaculata* and its habitat [30,31].

To summarize, the findings of this work describe differences in back spots between *S. infraimmaculata* populations in Israel affected by their different habitats. The population of salamanders in the Dan habitat where conditions are constant, and water is available year-round, is different from the two populations that come out in mountain habitats, with hot and dry summers and cold, rainy winters. Studies on the adaptation of *S. infraimmaculata* in Israel to different environments are supported by works from different fields of biology and typical to the genus *Salamandra*.

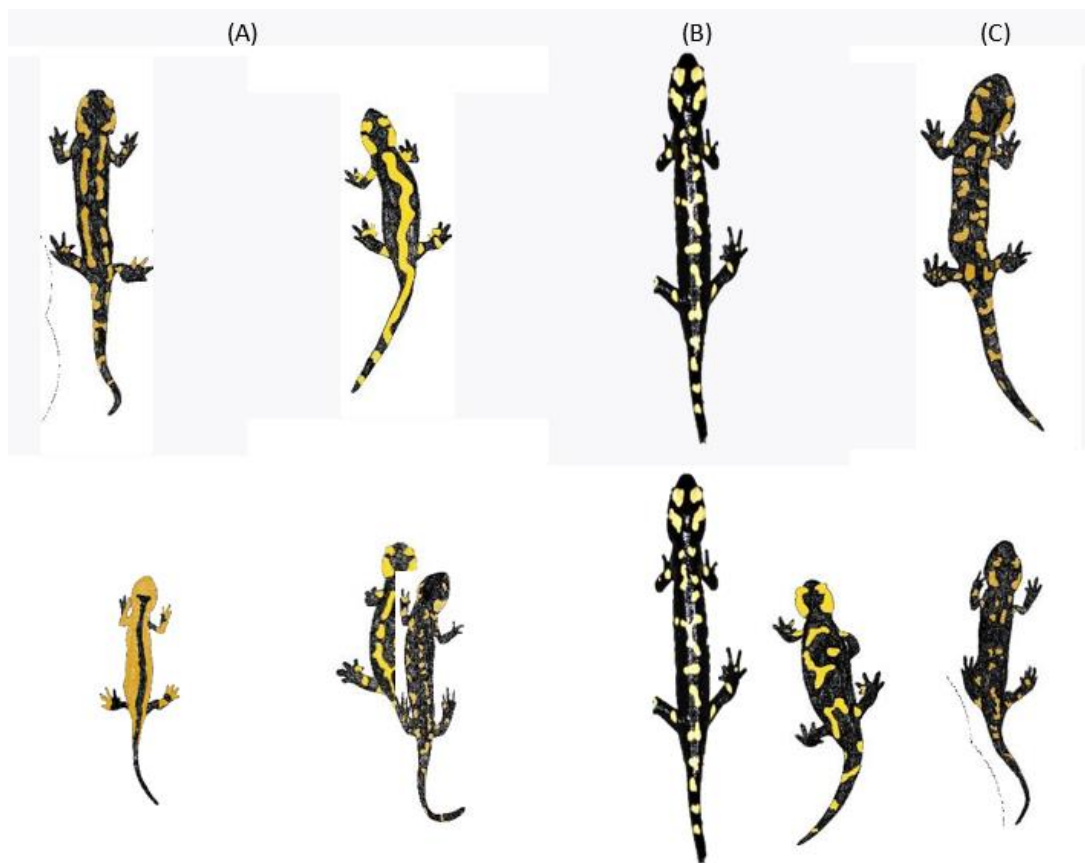


Figure 7: Color patterns in the various subspecies of *S. salamandra*. Some of these patterns also appeared in populations of *S. infraimmaculata*. (A) Spot patterns of *S. salamandra* that did not appear on *S. infraimmaculata* in present study. (B and C) Spot patterns of *S.*

Salamandra that also appeared on *S. infraimmaculata* in present study [5,6,29]. The images were entered into the computer and the percentage of yellow color was calculated as $[\text{Yellow}/(\text{Yellow} + \text{Black})] \times 100$.

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