SALAMANDRA **57**(4): 541–546
30 October 2021 | ISSN 0036–3375

SALAMANDRA
German Journal of Herpetology

Population size and habitat requirements of Derjugin's Mountain Newt (*Neurergus derjugini derjugini*) in Mirisour, western Iran

Pegah Karimi[1], Hanyeh Ghaffari[1,2] & Shahram Kaboodvandpour[1,2]

[1)] Environmental Sciences, Faculty of Natural Resources, University of Kurdistan, Sanandaj, Iran
[2)] Department of Zrebar Lake Environmental Research, Kurdistan Studies Institute, University of Kurdistan, Sanandaj, Iran

Corresponding author: Pegah Karimi, e–mail: karimi.pegah73@gmail.com

Manuscript received: 16 May 2021
Accepted: 3 October 2021 by Edgar Lehr

Abstract. Derjugin's Mountain Newt (*Neurergus derjugini derjugini*) inhabits the Zagros Mountains in northeastern Iraq and western Iran. The present study was conducted in Rikhalan village (Mirisour stream) in Marivan County, western Kurdistan Province, to determine habitat requirements and population size of Derjugin's Mountain Newt. From April through May 2020, the capture-mark-release-recapture technique was used to estimate population size. Habitat parameters including pH, dissolved oxygen and electrical conductivity of water, water and air temperature, the size of bedrocks, water flow speed, and canopy and aquatic vegetation cover were measured. A total of 3175 specimens of Derjugin's Mountain Newt (1853 males, 1322 females) were captured and recaptured. Population size was estimated using the software CAPTURE and the Jolley-Seber method. According to the results obtained by means of the MARK and Ecological Methodology software, the population size of Derjugin's Mountain Newt in the study area was between 2401 and 2554/1664 m^2, 1165 and 2877/1664 m^2, respectively. Habitat preferences of Derjugin's Mountain Newt in the Mirisour Stream are clear shallow water with a slow flow speed, pH 8.23–8.36, electrical conductivity 292–331 µS/cm, dissolved oxygen 7.26–8.42 mg/l, water temperature 12–17.5°C, and vegetation to include oak (*Quercus brantii*, *Q. infectoria*), plane (*Platanus orientalis*), walnut (*Juglans regia*), and willow (*Salix* spp.) while the aquatic vegetation consists of broadleaf (*Mentha* sp.) and orchard grass (*Dactylis glomerata*). Presence and abundance of this species were considerably higher at greater altitudes. The key threat to the species is habitat loss, which is caused by water being siphoned off for agricultural purposes and blocking springs as a result.

Key words. Caudata, Salamandridae Amphibian, population estimation, MARK and Ecological Methodology software, habitat variables.

Introduction

Amphibians are an integral part of many natural ecosystems. They serve as main prey for predators such as birds and reptiles across different ecosystems (Woolbright 1991, Duellman & Trueb 1994). Decreasing amphibian populations could have a major impact on natural ecosystems (Gardner 2001). Despite their ecological values, various anthropogenic factors currently affect their populations either directly or indirectly, and their populations are declining in many parts of the world. Amphibians, as much as other animal species, suffer from habitat destruction and fragmentation globally (Ashrafzadeh et al. 2019). Derjugin's Mountain Newt, *Neurergus derjugini* (Nesterov, 1916) inhabits the western margin of the Zagros Mountains in Iran and northeastern Iraq in a narrow distribution range. This species has two subspecies: Derjugin's Mountain Newt (*Neurergus d. derjugini*) and Small-spotted Mountain Newt (*Neurergus d. microspilotus*) (Safaei-mahroo & Ghaffari 2020). The IUCN Red List of Threatened Species classifies this species as 'critically endangered' (Safaei-mahroo et al. 2015).

A key parameter for evaluating the status of species conservation and the extinction risk level is population size (Segev et al. 2010). Population monitoring is one of the principles of wildlife management. Undoubtedly, the lack of information about population size, abundance and density of species impedes informed conservation management (Krebs 1989). Also, habitat variables need to be considered in management plans (Unglaub 2015). Population size (i.e., Minimum Viable Population) and habitat requirements are vital to safeguard the survival of endangered species. In the present study, the population size of Derjugin's Mountain Newt (*Neurergus d. derjugini*) was estimated based on the capture–mark–release–recapture method, and some variables in the aquatic and adjacent terrestrial habitats in Rikhalan village (Mirisour Stream) in Marivan County, western Kurdistan Province, were investigated.

Material and methods

Study site

This study was carried out in a stream called Mirisour, Marivan County, Kurdistan Province, in western Iran (35°26.4'38" N, 46°9.2'68" E) (Fig. 1). The climatic conditions of the study area are categorized as cold and humid with an average temperature of 12.8°C and the average annual precipitation amounts to 991.2 mm (Hanafi & Hatami 2013). The study site is located at an altitude of 1320–1555 m above sea level where the vegetation includes oak (*Quercus brantii*, *Quercus infectoria*), walnut (*Juglans regia*), and willows (*Salix* spp.).

Population estimation

Fieldwork was conducted from 22 April through 17 May 2020. To estimate the population size, capture–recapture sampling was performed once every five days by four persons in six surveys in which adult specimens were captured only. Population estimation was performed by means of the Jolly-Seber method in Ecological Methodology software (version 7.2). The study area was divided into three sections with lengths of 170 (the first station), 270 (the second station) and 600 metres (the third station), respectively. Sampling was always conducted by walking along the stream for 3 hours (from 11 through 14 h) (Safaei-mahroo et al. 2020). Specimens were captured by hand and were marked by toe clipping before being released in their original capture locations. During the first visit, the third toe of the left foot, in the second visit, the third toe of the right foot, in the third visit, the second toe of the left hand, in the fourth visit, the second toe of the right hand, and in the fifth visit, the first toe of the left foot were cut off. The numbers of new captures and recaptures were recorded on each occasion (Table 1). In Table 1, only recaptures with one mark were recorded in the recapture column and samples with two or more marks were considered as total recaptures. Therefore, to estimate the population size of Derjugin's Mountain Newt, those estimates were considered that had the highest ratios of markers and the lowest standard errors. Accordingly, the estimated population values were tabulated (Table 2) and then each station was considered a replication, for which averaging was performed. To estimate the total population size of adult newts, a closed population was designated. We then applied the software CAPTURE in MARK (version 9.0) (Otis et al. 1978) and the Jolley-Seber method in Ecological Methodology software. To choose the best model for estimating population size, the Goodness of fit method in MARK software was used (O'Donnell et al. 2008). As each of these population estimation models has an error if the value of p_ hat (probability of capture) is equal to or greater than 0.1 and the value of t (number of trapping occasions) is equal to or above 5 ($p \geq 0.1$, $t \geq 5$), the bias due to population size estimation by the models can be neglected (Otis et al. 1978). According to Table 3, the value of p_ hat is equal to 0.2, and t is equal to 6. Selecting and testing the appropriate model for capture and recapture methods in closed populations is still an unresolved issue (Cooch & White 2014), though. If only one of the variables, such as time, behaviour or heterogeneity, is considered to be effective in capture probability, then Goodness of fit can be considered a suitable model for testing the Mo, Mt, and Mb models. As suggested by Table 4, model Mo vs. Mt, means that time is the factor of change in the probability of catching and model Mo vs. Mb means the behaviour of the factor of change in the probability of capture.

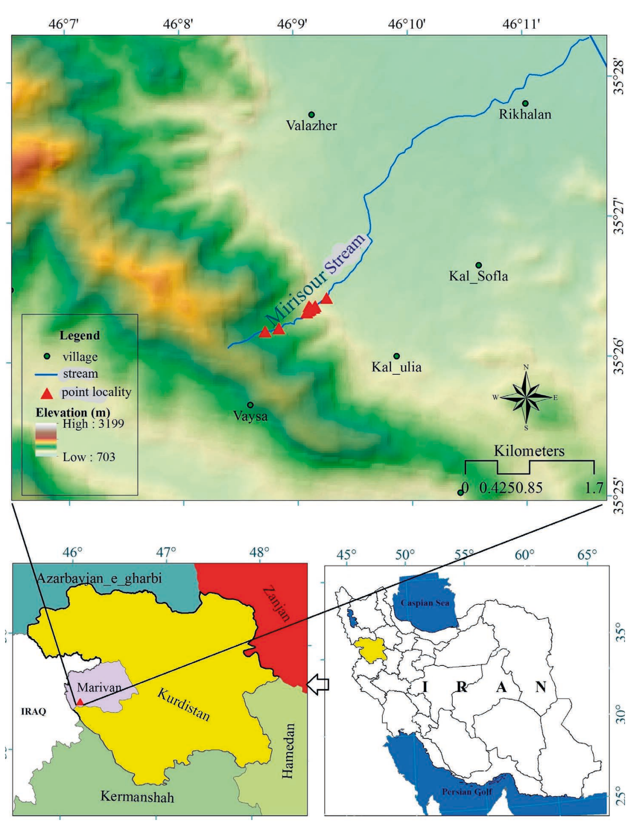

Figure 1. Location of the study area.

Habitat variables

As a part of the study, the stream over a length of 1109 m was selected as a macrohabitat to measure the variables of aquatic habitat. Then, this section was divided into three consecutive stations as microhabitats to determine the variations between habitat characteristics and the relationship between species density and habitat characteristics. In our field surveys, habitat variables including water temperature and air temperature, measured by glass thermometer, electrical conductivity, acidity and dissolved oxygen, through the multi-parameter device (model: HACH, HQ40d), altitude, stream area and length and latitude, by the GPS device (model: GARMIN GPS MAP 78S), slope percentage (Compass software) and water flow velocity were measured and recorded. To determine the water flow veloc-

Table 1. Counts of new captures and recaptures in the first to the sixth samplings.

Station	Date	Total captures	New captures	Recapture 1	Recapture 2	Recapture 3	Recapture 4	Recapture 5
1	22/4/2020	72	–	–	–	–	–	–
2	22/4/2020	168	–	–	–	–	–	–
3	22/4/2020	382	–	–	–	–	–	–
1	27/4/2020	77	46	31	–	–	–	–
2	27/4/2020	135	77	58	–	–	–	–
3	27/4/2020	227	188	36	–	–	–	–
1	2/5/2020	73	34	16	11	–	–	–
2	2/5/2020	115	50	30	19	–	–	–
3	2/5/2020	343	225	70	38	–	–	–
1	7/5/2020	63	24	10	4	10	–	–
2	7/5/2020	136	50	30	20	8	–	–
3	7/5/2020	294	183	37	34	19	–	–
1	12/5/2020	44	12	2	–	6	6	–
2	12/5/2020	118	24	19	13	11	16	–
3	12/5/2020	339	161	31	26	40	24	–
1	17/5/2020	27	7	2	2	–	2	2
2	17/5/2020	105	18	11	6	7	6	6
3	17/5/2020	443	169	32	29	33	35	39

Table 2. Population size at each station as estimated with Ecological Methodology software.

Station	Estimate±SE	Proportion marked	Lower 95%	Upper 95%
1	389±91.3	0.41	269	715
2	1129±455.7	0.5	628	2822
3	3393±575.4	0.35	2600	5095

Table 3. Population size estimate for Derjugin's Mountain Newt in the study area with a total area of 1664 m². t = number of trapping occasions, Mt+1 = number of animals captured, P–hat = estimated probability of capture, CI = an approximate 95% confidence interval.

Model	Estimator	t	Mt+1	P–hat	Estimate±SE	95% CI
M0	–	6	1892	0.2136	2477±39.38	2406–2560
Mt	Chao	6	1892	0.2066	2536±54.86	2437–2652
Mt	Darroch	6	1892	0.2133	2472±39.10	2401–2554
Mb	–	6	1892	0.2192	2445±66.68	2330–2592

Table 4. The model selected by the Goodness of fit method.

	M0 vs. Mh	M0 vs. Mt	M0 vs. Mb
Chi_ Square	5.47	67.4	0.3
Degrees of freedom	3	5	1
Sig	0.14	0.000	0.56

Table 5. Criteria for model selection by the Goodness of fit method.

Model criteria	M0	Mt	Mb
	0.12	1	0.13

ity, two points between a certain distance were selected. After measuring the distance between these two points (in m), a piece of wood was dropped into the water at the upstream point and the time required for it to reach the downstream point was taken with a stopwatch. Finally, the measured distance was divided by the time recorded and water flow velocity was calculated based on m/s (Allan 2007). It should be noted that the velocity of water flow was measured at several points and extrapolated to apply to the whole stream. Water depth was also measured at various points by a metal measuring tape. Also, in each station, the type and percentage of vegetation inside and outside the water, the percentage of the canopy cover was recorded, and vertebrate (Safaei-mahroo & Ghaffari 2020) and invertebrate (Clifford 1991) species coexisting with Derjugin's Mountain Newt were noted.The bedrocks of the water course were categorized based on the method suggested by Sadeghifar & Azarmsa (2016), i.e., bedrocks were classified into four different categories: pebble (4–64 mm in grain size), cobble (64–256 mm), boulder (> 256 mm), and sand-clay (particles < 2 mm). To evaluate the differences between physical and chemical factors of the water and the difference in altitude between the three stations, one-way ANOVA, and to investigate the relationship between habitat variables and species density at each station, a Pearson correlation test (Pearson's correlation coefficient) were performed using SPSS software (Version 16.0).

Table 6. Results of the amount of environmental, physical and chemical factors at each station by one-way ANOVA.

Station	Water temperature	Air temperature	DO	pH	EC	Altitude
1	13.9±1.7	20.3±1.8	7.89±0.28	8.31±0.8	296.9±10	1335±11
2	13.3±1.5	19.9±1.7	8.44±0	8.37±0.8	309.6±12	1385±12
3	13.8±1.7	19.6±1.5	7.56±0.18	8.20±0.1	336.4±14	1554±18

Results

Population estimation

During our field surveys, a total of 3175 adult Derjugin's Mountain Newts were captured (Table 1). Based on the obtained results, with a 95% confidence interval, its population was estimated to be between 1165 and 2877 individuals in the study area of 1664 m². Furthermore, the population size was estimated with the MARK software (Mt, Mb and Mo estimators in CAPTURE) (Table 3) at 2500/1664 m² in the study area. Based on the obtained results, Mt (Darroch) model turned out to be the best model for estimating population size (Table 4). According to the Mt (Darroch) model, the population size of Derjugin's Mountain Newt in the study area with its area of 1664 m² was estimated with a 95% confidence interval to be 2401–2554 individuals, and the density was 0.16 to 0.17 individuals/m². The total area of the stream was 14,486 m². Given the fact that the species was observed only at altitudes above 1370 m , this estimate could be extrapolated to the entire water area. Thus, according to the Ecological Methodology, the population size of Derjugin's Mountain Newt in the entire water body with its area of 14486 m² was 1165 to 2877 and the density was 0.08 to 0.2 individuals/m². According to these results, Mt is the only model that is significant compared to Mo ($P < 0.05$), and it may therefore be concluded that time is the only source of change in the probability of capture and Mt can be used as a suitable model for population estimation. The results of the Goodness of fit model (Table 5) also indicate that the Mt (Darroch) model is the best model for estimating population size, because it has the highest value compared to the other two models.

Habitat Requirements

The results of a one-way ANOVA indicated that among the water physical and chemical factors, acidity and electrical conductivity differed significantly between the three stations. There is also a notable difference between stations in terms of altitude (Table 6).

According to the results of the Pearson correlation test, the correlation coefficient between acidity and dissolved oxygen and the density of specimens was negative (pH = -0.89, DO = -0.98). The relationship between pH and the density was not significant at a level of 0.05 ($P > 0.05$, Sig = 0.3). The relationship between DO and density was not significant at a level of 0.05 ($P > 0.05$, Sig = 0.1). Electrical conductivity had a positive correlation coefficient (EC = 0.98) with density, but this relationship was not significant at a 0.05 level ($P > 0.05$, Sig = 0.1). The correlation coefficient between altitude and density was positive (= 1), that is, density increases with increasing altitude, and the relationship between these two variables was significant at a level of 0.05 ($P < 0.05$, Sig = 0.009). Therefore, the values of acidity, electrical conductivity, dissolved oxygen, and water temperature at the three studied stations are slightly different from each other yet have no effect on the density of samples in the selectedsectors. The altitude variable, which was increasing from Station 1 to Station 3, proved to be an important factor determining the density of the Derjugin's Mountain Newt in its habitat in the MirisourStream.

Water flow velocity was measured to be between 0.2 and 0.5 m/s, and water depth veried between 2 and 67 cm. The stream's bedrock was composed of 40% pebble (4–64 mm), 25% cobble (64–256 mm), 15% boulder (> 256 mm), and 20% sand-clay (particles < 2 mm). It was found that the species occurred in higher density in areas where the proportional contents of pebble and sand-clay were larger. The dominant vegetation in the study area was oak (*Quercus brantii*, *Q. infectoria*), plane (*Platanus orientalis*), walnut (*Juglans regia*), and willows (*Salix* spp.), and aquatic plants were broadleaf (*Mentha* sp.) and orchard grass (*Dactylis glomerata*). In some parts of the water course, bryophytes were also observed (Fig. 2). Aquatic vegetation was sparse (30%) and vegetation debris covered a significant percentage of the streambed. Due to the dark brown colour pattern of Derjugin's Mountain Newt larvae, brown accumulations of leaf litter are a good place to find refuge. This species spawns on submerged branches (Fig. 3). The species coexisting in the same habitat included Bedriaga's marsh frog (*Pelophylax bedriagae*), freshwater crab (*Potamon* sp.), and insect larvae of the families Gyrinidae, Geriidae Heptageniidae, Stratiomyidae, Tipulidae and Zygoptera.

Discussion

This study and previous research (Mirani 2013) have found that the best time to study the population size of adult Derjugin's Mountain Newt is in the reproduction season from early April through late May, a period during which both males and females are present in the water. According to studies conducted by Feizi & Ezzati (2019), who focused on the habitat status and population of the species in the Mirisour Stream, the population size estimated by the Lincoln-Petersen method was about 1000 in an area of 1120 m². Our results indicate that the topographic characteristics of the terrestrial habitat of Derjugin's Mountain Newt around the stream are a steeply sloped forest habitat with a sparse covering of oak trees in grassland. This species was present at altitudes above 1,000 m, and its density and presence increased with increasing altitude. Based on the studies of Afroosheh et al. (2016), which examined the distribution of the Yellow-spotted Newt (*Neurergus d. microspilotus*) in western Iran, all localities of this taxon ranged between 630 and 2057 m above sea level. The terrestrial habitats adjacent to the streams ranged from treeless floating meadows to dense oak forests. In the study area, the values of pH ranged from 8.36 to 8.23, electrical

Figure 2. Aquatic and terrestrial vegetation: the upper image shows aquatic plants, 22 April 2020, and the lower image shows trees around the stream, 17 May 2020.

Figure 3. Camouflaged larvae and spawn on submerged branches, 17 May 2020.

Figure 4. A water pipe draining the stream source for agricultural irrigation.

conductivity was somewhere between 292 and 331 μS/cm, dissolved oxygen registered at 7.26–8.42 mg/l, and water temperature varied between 12.5 and 17.5°C. According to Sharifi & Assadian (2004), who examined the distribution and conservation status of the Yellow-spotted Newt in a stream in Qory Qala in Kermanshah Province, electrical conductivity was between 323 and 356 μS/cm, pH was at 6.65–8.15, and water temperature was reported to be 11°C. Lowe et al. (2012) examined the physical and chemical factors of the aquatic habitat of *Gyrinophilus porphyriticus* in North America, where the pH was reported to be 6–5, electrical conductivity ranged between 12–15 μS/cm, and temperature was between 13 and 17°C. The bed of the studied aquatic habitat was mainly composed of pebble and cobble with 1.5–250 mm in grain size. Sharifi & Assadian (2005) examined the bed of the Kavat River in Kermanshah Province, which had the highest number of Yellow-spotted Newts. They recorded the size of its cobble as 2–256 mm. Our results indicate that Derjugin's Mountain Newt occurs at its highest density at higher altitudes in the study area. Habitat preferences of Derjugin's Mountain Newt in the Mirisour Stream are clear shallow water with a slow flow and good oxygenation, pH 8.23–8.36, electrical conductivity 292–331 μS/cm, dissolved oxygen 7.26–8.42 mg/l, water temperature 12–17.5°C, sparse aquatic vegetation, bed material with a high content of sand-clay and low content of cobble, and a dense canopy cover. The species is more abundant on the margins and floors of shallow areas, where the water flow is slow. Based on Safaei-Mahroo & Ghaffari (2020) and our observations, the main threat to the species is habitat loss imposed by water being siphoned off for agricultural irrigation in a manner that has effectively blocked the springs (Fig. 4).

Acknowledgements

We greatly appreciate the kind help and advice of B. Safaei-Mahroo. We are very grateful to E. Karimian and S. Adak for their valuable support during our field studies. We do also express our gratitude to N. Hoseini, who guided us through the study area.

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