Online ISSN: 2588-3526

Journal of Wildlife and Biodiversity

Volume 5 (4): 68-81 (2021) (http://www.wildlife-biodiversity.com/)

Research Article

Comments on the distribution and population estimation of *Neurergus derjugini* (Urodela, Salamandridae) in western Iran

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Received: 15 May 2021 / Revised: 14 July 2021 / Accepted: 17 July 2021 / Published online: 09 December 2021. Ministry of Sciences, Research, and Technology, Arak University, Iran.

How to cite: Heidari, N., Hosseinian Yousefkhani, S.S., Faizi, H. (2021). Comments on the distribution and population estimation of *Neurergus derjugini* (Urodela, Salamandridae) in western Iran. Journal of Wildlife and Biodiversity, 5(4), 68-81. **DOI: 10.22120/jwb.2021.130245.1227**

Abstract

Basic knowledge of population status is effective for the conservation of many species, especially threatened and endangered species. In this study, we used the Mark-recapture technique (Lincoln-Petersen method) as an established method for estimating population in wildlife surveys. Population surveys of critically endangered newts conducted for *Neurergus derjugini* in the streams of Kurdistan and Kermanshah Provinces, western Iran, at Sarvabad, Marivan, Baneh, Saghez, and Nosoud. The study consists of estimating population size by sampling annually for two consecutive years with each annual sampling consisting of several surveys between May-August. The species has a restricted distribution, occurring at high mountain streams. Our results also reject the occurrence of this species in Sarvabad and Awraman stream habitats and water ecosystems as was stated by former researchers. Distribution information of this species was updated in the region, and all known records are mapped. The results showed that Miri Sour (Marivan) with 1591-1568 and Asnabad (Marivan) with 14-18 specimens as estimated populations were found as most and less populated and suitable habitats respectively.

Keywords: Kurdistan, Kermanshah, mountain newt, Mark-recapture technique, Petersen method

Introduction

Understanding the distribution and abundance of organisms is usually considered as the main goal of ecological considerations (Elton 1927, Krebs 1972). Basic knowledge and having relatively accurate information on the population status of a species is effective for conservation and management of the species, especially is much more important and valuable in critically endangered, threatened, and endangered species. *Neurergus derjugini* considered a critically endangered species (IUCN 2010) is subjected to considerable studies such as phylogeny (Steinfartz et al., 2002; Rancilhac et al., 2019; Afroosheh et al., 2019; Malekoutian et al., 2020), distribution pattern (Najafimajd & Kaya, 2013; Afrooshe et al., 2016), ecology and conservation (Sharifi & Afrooshe, 2014; Rastegar Pouyani et al., 2013) and taxonomic accounts and revisions (Hendrix et al., 2014; Bozorgi et al., 2015) but little is known about the population status of this species of conservation concern.

Estimate of abundance in the mountain newt (*Neurergus derjugini*) is strongly dependent on seasonal streams and water ecosystems during early spring when the species passes its breeding and resting. This means that seasonal streams of the area may not only impress breeding success of the species populations but may also affect the census process and monitoring program of the species conservation. The species live in freshwater mountain stream habitats, but habitat degradation and reduction of dispersal activities due to patched habitats are among the main threats to the small endemic populations of the species (Rastegar Pouyani et al., 2013; Afrooshe et al., 2016). The recent genetic studies point out a high genetic diversity among the populations of the current taxa of the genus *Neurergus* and concluded current taxonomy of the current species might underestimate the species richness and subdivided into further species and subpopulations inhabiting different parts of each species distribution range (Gül, 2019; Rancilhat et al., 2019; Kurnaz & Şahin 2021). Kurdistan's salamander fauna consists of two-three species dependent on the taxonomic schemes (Bahmani et al., 2014). These include *Neurergus microspilotus*, *Neurergus* cf. *derjugini*, and *Salamandra infraimmaculata semenovi*. Since the Nesterovs expedition 1914, discovered *Neurergus derjugini* from Ghorighaleh, Kermanshah, a series of records on distribution of the critically endangered Kurdistan spotted newt, *Neurergus derjugini* (Nesterov 1916) has presented so far (Najafimajd & Kaya 2010; Schneider & Schneider 2011; Fadhil et al., 2013; Afroosheh et al., 2016; Afroosheh et al., 2019; Malekoutian et al., 2020).

Neurergus derjugini is considered a Critically Endangered (CR) species and is found in Iran at the border with Iraq (IUCN 2010). The species is not listed under the CITES database but it systematically is under illegal trading and needs to monitor in the area. In this study, we make the case study of a population census in *Neurergus derjugini* using the mark-recapture technique (Petersen method) and estimate the closed population size at 11 examined localities visited during 2020- 2021 for both scientific and conservation perspectives as reasonable state variables for investigation.

Material and Methods

Species and Study area

This study was performed at Kurdistan (Sarvabad, Marivan, Saghez, and Baneh) and Kermanshah (Nosoud) provinces (Table 1 and Fig. 1). Visits have been done between June-August 2020-2021 during the breeding seasons as the most appropriate season for samplings and census population size in natural populations. As the first stage of the study (capture, marking, and realizing the specimens), 11 expeditions lasting one day each, were conducted to the target sites of the study area for capture and marking of the specimens (Fig. 2). Then we performed the second stage of the study (recapture and complete the estimation parameters) during the same time (June-August 2019). Our samplings took place between 10:00-13:00. We just searched and collected both adults and sub-adults/juveniles and no egg clutches were found and counted. In this study, we attempted to census the population of the species using the mark-recapture technique (Petersen method) involves drawing a random sampling, marking the specimens, releasing them, and finally recapture the specimens. The toe clipping method is one of the common marking methods (Waichman, 1992**)** used in this study. To better specimen tracking of animals, a trifling patch of toe apex has been taken and due to rapid regeneration of toes in newts (Henle et al., 1997), the recapturing of specimens had been conducted immediately after 15 days of capturing and marking the specimens. In three-hour transects along streams, we employed Visual Encounter Surveys (VES) with toe-clipping as the primary technique of mark and recapture. It should be noted that we had completely identical conditions for the first and second round in terms of the number of participants in each sampling series (three specimens for each round), the length of the river surveyed (approximately 800 m of river flow), the time taken to perform sampling and search patrols (approximately three hours) and the length of day during which sampling took place (10:00-13:00). As a result, every effort was made to get the first and second samplings under the same conditions. Furthermore, 15 days after the initial sampling and labeling of the captured specimens, these specimens have had almost enough time to combine with other populations in their habitat and to restore their populations to normal conditions.

Table 1. List of the visited localities for *Neurergus derjugini* in western Iran

Row	Stations	Address	Coordinates	Elevation (m)	Figures
1	Miri Sour	Kordestan province, Marivan	35.439865°- 46.153194°	1370	Fig. 4A
2	Dirgashekhan	Kordestan province, Marivan	35.410655°- 46.196490°	1400	Fig. 4B
3	Pirankohan	Kordestan province, Marivan	35.499611°- 46.028975°	1460	Fig. 4C
4	Dolabi	Kordestan province, Marivan	35.523776°- 46.033011°	1520	Fig. 4D
5	Asnabad	Kordestan province, Marivan	35.562679°- 45.997980°	1400	Fig. 4E
6	Wlajher	Kordestan province, Marivan	35.460576°- 46.141731°	1380	Fig. 4F
7	Gholghola	Kordestan province, Saqez	36.052925°- 46.034819°	2100	Fig. 4G
8	Garmab	Kordestan province, Baneh	35.912444°- 45.723357°	1300	Fig. 4H
9	Sonch	Kordestan province, Baneh	36.046584°- 46.004396°	2000	Fig. 4I
10	Kanibard	Kordestan province, Baneh	36.061462°- 45.643328°	1350	Fig. 4J
11	Najjar	Kermanshah province, Nosoud	35.095714°- 46.322947°	1350	Fig. 4K

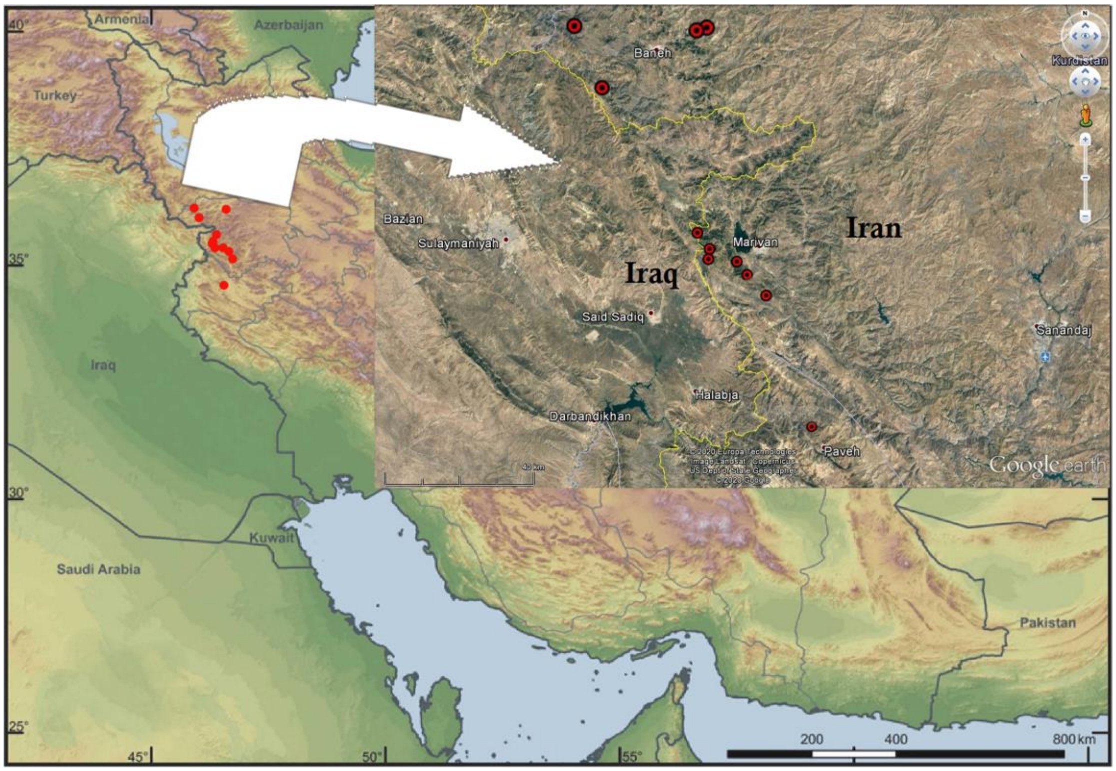

Figure 1. Eleven visited localities for *Neurergus derjugini*

Figure 2. Dorsal and ventral views of *Neurergus derjugini* (Locality: Marivan, Miri Sour)

Data analyses

The Lincoln-Petersen method has been widely used for its simplicity (John and James 1988, Amstrup et al., 2006 & Jeffrey et al., 2010) because we only have one marking and one retrieval. This is a way of marking several specimens for a short time, releasing them, and then retrieving them and checking their marks. The second sampling for this method was in the same condition as random sampling to validate the method. Therefore, all specimens had the same chance of being caught in the second sampling. Depending on whether they are marked in the second stage, the following data were obtained:

M = is the number of captured and marked specimens at the first sampling time

C = is the number of caught specimens at the second sampling time

R = is the number (of those C animals) that had a mark

Based on these three variables, an estimation was obtained:

$\hat{N}$ = is the estimated population size at the time of marking

with a simple ratio we obtained the following equation:

$$\frac{N}{M} = \frac{C}{R} \Rightarrow \hat{N} = \frac{CM}{R}$$

This is a closed method, and our sampling and estimation also met the criteria of a 'closed' population model (which assumed no births, deaths, immigration, or emigration from the population during the study) (Krebs, 1998). Unfortunately, this formula has a major source of bias that it represents more than the actual population, which can be very large for small populations. That is if there is $(M + C)\rangle N$, the equation is unbiased, and also if at least 7 marked specimens are recaptured in second sampling ($R\rangle 7$), the equation is unbiased.

Results and Discussion

Mark-recapture techniques were used to generate population estimates for 11 study sites, one site in Kermanshah province, and 10 sites in Kurdistan province (Table 1).

Totally 2437 and 2512 juvenile/adult specimens (as the sum of M and C values at above equation) distributed in 11 visited localities were collected during 2020 and 2021 respectively. Of these, 1072 and 1148 specimens (51.4% and 49%) belong to the first capturing stage and 1013 and 1194 specimens (48.6% and 50.9%) belong to the second capturing phase during 2020 and 2019 respectively. Yearly capture and recapture numbers are summarized in Table 2. The lowest population estimate was 14 newts in Asnabad in 2021 and 10 newts in Kanibard in 2021, and the highest estimate was 1591 and 1568 newts in Mirisour in 2020 and 2021 respectively (Table 2 and Fig. 3). Because the sex of newts could not be determined, the data include both males and females.

Table 2. The number of newts estimated from captures. M, number of marked specimens in the first sampling; C, the total number of collected specimens in the second sampling; R, the number of specimens in the second sampling who were symptomatic; estimated population size (population estimate (Petersen method) calculated from recapture rates).

Parameters	**2020**				**2021**			
Station	**M**	**C**	**R**	$\hat{N}$	**M**	**C**	**R**	$\hat{N}$
Miri Sour	550	512	177	1591	561	520	186	1568
Dirgashekhan	85	74	55	114	96	82	64	123
Pirankohan	124	118	98	149	135	126	107	159
Dolabi	114	100	120	95	125	254	129	246
Asnabad	21	27	12	18	16	18	21	14
Wlajher	48	40	10	192	59	48	19	149
Gholghola	25	18	7	64	36	26	16	59
Garmab	42	35	23	64	38	32	21	58
Sonch	24	30	14	51	35	38	20	67
Kanibard	21	32	18	37	18	15	27	10
Najjar	18	27	8	61	29	35	17	60
Total	**1072**	**1013**	**542**	**2437**	**1148**	**1194**	**627**	**2512**

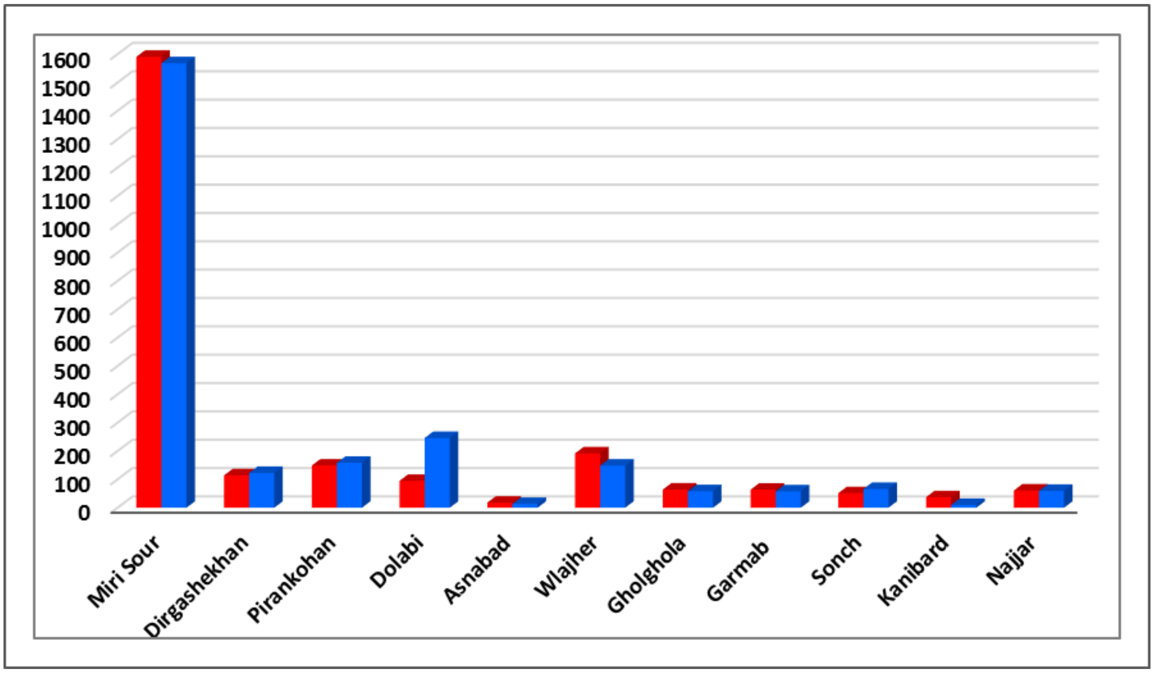

Figure 3. Estimated population size ($\hat{N}$) for the target species at two consecutive years 2020 (red bars) and 2021 (blue bars) at visited sites


Figure 4A


Figure 4B


Figure 4C


Figure 4D


Figure 4E


Figure 4F

Figure 4G

Figure 4H

Figure 4I

Figure 4J

Figure 4K

Figure 4. Some visited and studied habitats during the two-year study period in Kurdistan and Kermanshah provinces. The habitats are listed in Table 1.

Phylogeny, taxonomy, and ecology of the species have long been considered elsewhere (Steinfartz et al., 2002; Rancilhac et al., 2019; Najafimajd & Kaya, 2012; Afrooshe et al., 2016; Bozorgi et al., 2015) however, this study provides complementary data on quantity, abundance and population census of the species after Afrooshe et al., (2016). The occurrence of these species from “Zhiwar, Naw, Novin, and Selin” (Afrooshe et al., 2016) that their occurrence was contradicted to our expectation was confirmed rejected in this study since these localities host another salamander (*Salamandra infraimmaculata semenovi*). This species is reported as new for many territories of western Iran and discoveries on the occurrences from Marivan and Baneh expand *N. derjugini* distribution range. The presence of this species in some formerly recorded localities and many new records confirmed, mapped, and documented in this study. The new records expanded the geographic range distribution of this species, but on the other hand while rejecting previous data-limited its range. Further research in the province has discovered several additional locations for *N. derjugini* in the Marivan and Baneh districts, near Zariwar Lake upstream of the lake basin. These new localities are presented in Table 1 and Figure 1. Based on provided records of available literature (Najafimajd & Kaya 2010; Bahmani et al., 2014; Bozorgi et al., 2015, Schneider and Schneider 2015, Barabanov and Litvinchuk 2015 & Afroosheh et al., 2016) and result of our surveys, the updated geographic distribution map of this species present the known exact records at western Iran.

The number of specimens and as a result estimated population at the stations of Miri Sur at the Marivan area in both consecutive years were better and the habitats were more favorable than all other studied habitats. Due to the seasonality and drying up of habitats at least three to four months of the year, mid-summer to early autumn (August to October), this habitat becomes completely dry and there is no evidence of their presence or occurrence in the habitats. The rest of the studied habitats are relatively similar and have smaller populations than those mentioned. It should be noted, however, that all other habitats also lack water and environmental flow during the dry and low rainfall months (August to October).

The species was least detectable during the summer months and most detectable during the spring months. Their absence during dry and warm months is mostly due in part to low energetic requirements of amphibians, allowing them to spend prolonged periods underground or undercover (Petranka,1998), with activity limited to periods of ideal biotic conditions (Connette et al., 2011 & Steven et al., 2011). The mark-recapture method in estimating the population status of the target species was ideal since the mobility of the species was limited, though the accurate estimation is often difficult because the species is cryptic. The techniques we used in mark-capture-recapture of newts are widely used in amphibian population studies (Bailey et al., 2004a, Cecala et al., 2009 & Grant et al., 2010; Connette & Semlitsch 2015; Worthington et al., 2019).

One of the main threats of the Gholghola station is the construction of a gold mine upstream of the habitat, which in the future will cause many problems for habitat contamination. Based on our observations, experience, and local knowledge, the species has declined in its distribution range and habitats in the past two decades. However, the extent of the species population declines is unknown. One of the dispersal characteristics of these newts in nature is the discrete and fragmented populations, which due to their poor ability to inherently and actively distribute from one point to another, so very little genetic exchange between different populations of this species is possible (Stuart et al. 2008). Known from few remaining streams, in fragmented habitat; there is no gene flow between the populations. This has led to an increase in intra-population gene exchange and a decrease in inter-population gene exchange, thereby drastically reducing genetic diversity in populations, which has led to severe risks for different populations of this species locally and nationally (Stuart et al. 2008). Due to this fact, *Neurergus derjugini* has experienced a severe population decline in rivers and streams of the area.

This newt has attracted serious interest from traders in both local and international markets due to its unique delicacies. Therefore, in addition to other environmental concerns encountered in nature for these animals, they have come to threaten even more (Rastegar-Pouyani 2003; Sharifi et al. 2008). *Neurergus derjugini* has long been extinct from its main and important habitats in Kermanshah province, especially within the type locality of Ghorighaleh (Sharifi et al. 2008). Drought, excessive water harvesting, pollution of water resources, increased agricultural activities, and other human activities in many areas within the range of this species distribution or on the margins of their distribution where current waters enter aquatic habitats of this species all have an impact on the remaining populations of this species (Sharifi & Assadian 2004).

Protecting the habitat of this species will not only preserve the ecological conditions for hundreds of other species of aquatic invertebrates and ecosystem health but will also protect and conserve water resources for local populations in remote areas (Sharifi and Assadian 2004; Rastegar-Pouyani 2006). In addition to recent droughts that have reduced water resources in habitats in most parts of the country, water resources contamination especially in adjacent habitats in rural areas and small towns, due to chemical contaminants such as insecticides, various pesticides, and fertilizers have had a significant role in reducing the population of these species in different areas of their distribution range (Sharifi et al. 2008). Further studies on habitat and distribution modeling of this species under different climatic scenarios in the next few decades will provide more and more important information about the status of this species in the future by combining these modeling data with the current situation of the species, to make better decisions on its conservation.

Acknowledgment

The author is thankful to “The Ocean Park Conservation Foundation (OPCF)”, Hong Kong authorities for their financial support and technical/logistic collaborations with the Department of Environment during the study period.

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