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Estimating the Population Size of *Neurergus kaiseri* (Caudata: Salamandridae) in Iran: Comparison of Estimation Methods Provides Implications for Conservation

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***Abstract.*—We estimated the size of populations of Kaiser's Mountain Newt (*Neurergus kaiseri*) occupying habitat types representative of its range to provide baseline information for future monitoring and status assessments. We selected three distinct but representative breeding habitats in the mountain forests of southwestern Iran to compare the Lincoln Peterson, Schumacher-Schnabel, and Jolly-Seber methods used to estimate population size. We recommend using the Jolly-Seber method during the breeding season (15 April-15 May) with three sampling sessions, each separated by four-day intervals. Using this method, we estimated Kaiser's Mountain Newt population size at 1,403 individuals in the Bozorg-Ab habitat, 491 in Dej habitat, 63 in Alan Spring, and 321 in Monjir Spring within the Barik-Ab habitat. We provide a guideline for estimating population and monitoring mountain newt populations.**

Key Words.—capture-mark-recapture method; closed population estimation; endemic; Middle East; open population estimation; sex ratio

Introduction

Understanding size and demography of wildlife populations is essential for species conservation programs (Selman and Jones 2017). Such population data are scarce for endemic, vulnerable, and endangered amphibian species in the Middle East. The International Union for Conservation of Nature (IUCN) Red List has been an important resource for assessing the global conservation status of species; however, rare, endemic, or understudied species may lack sufficient data to critically evaluate their conservation status, and to detect declines in either populations or species (Geyle et al. 2018, 2021). Hence, estimates of population size are essential for establishing and evaluating the conservation status of rare or endemic species, and for developing conservation management practices.

Twenty-two species of amphibians have been identified in Iran, including 13 genera from seven families, in either the Order Anura (frogs and toads) or Order Caudata (salamanders and newts). Iranian Caudata are restricted to the mountainous regions of Alborz and Zagros forests. True salamanders (Salamandridae) and Asiatic salamanders (Hynobiidae) comprise nearly one third (32%) of Iranian amphibian biodiversity (Safaei-Mahroo 2014; Ghaffari et al. 2020). Only four species of *Neurergus* have been identified worldwide, and all of them are endemic to the Tigris-Euphrates watershed in the Zagros and Anatolian Mountain ranges of Iran, Iraq, and Turkey (Safaei-Mahroo and Ghaffari 2021).

Kaiser's Mountain Newts (*Neurergus kaiseri*) are endemic to Iran and reported from only a few patches of oak forests dominated by the trees Oak Manna (*Quercus brantii*) - Mount Atlas Pistache (*Pistacia atlantica*), and *Q. brantii* in mountainous and semi-mountainous areas mainly formed by Pliocene Bakhtyari conglomerates from the south of Lorestan Province northward to North-West Khuzestan Province (Safaei-Mahroo 2014). This species inhabits mountain streams, springs, and little arroyos from 400 to 1,619 m elevation in the Karkheh and Great Karun basins. It prefers aquatic habitats with a depth of 30 to 150 cm, and temperatures from 15° to 21.5° C (Safaei-Mahroo 2014). The breeding season varies based on latitude and elevation, and adults can be found breeding in springs and streams from mid-March to the end of May. Although mostly nocturnal, *N. kaiseri* are also active during the day while breeding. Adults are relatively slow-moving and, therefore, easy to capture by hand during the mating season. The dorsal color pattern of *N. kaiseri,* black ground color with large white blotches and an orange vertebral stripe (Fig. 1), makes it easy to distinguish from congeners (Safaei-Mahroo and Ghaffari 2020).

Illegal collection for the pet trade is the main threat to *N. kaiseri*, which is the only species of Iranian amphibian listed on the Convention on International Trade in Endangered

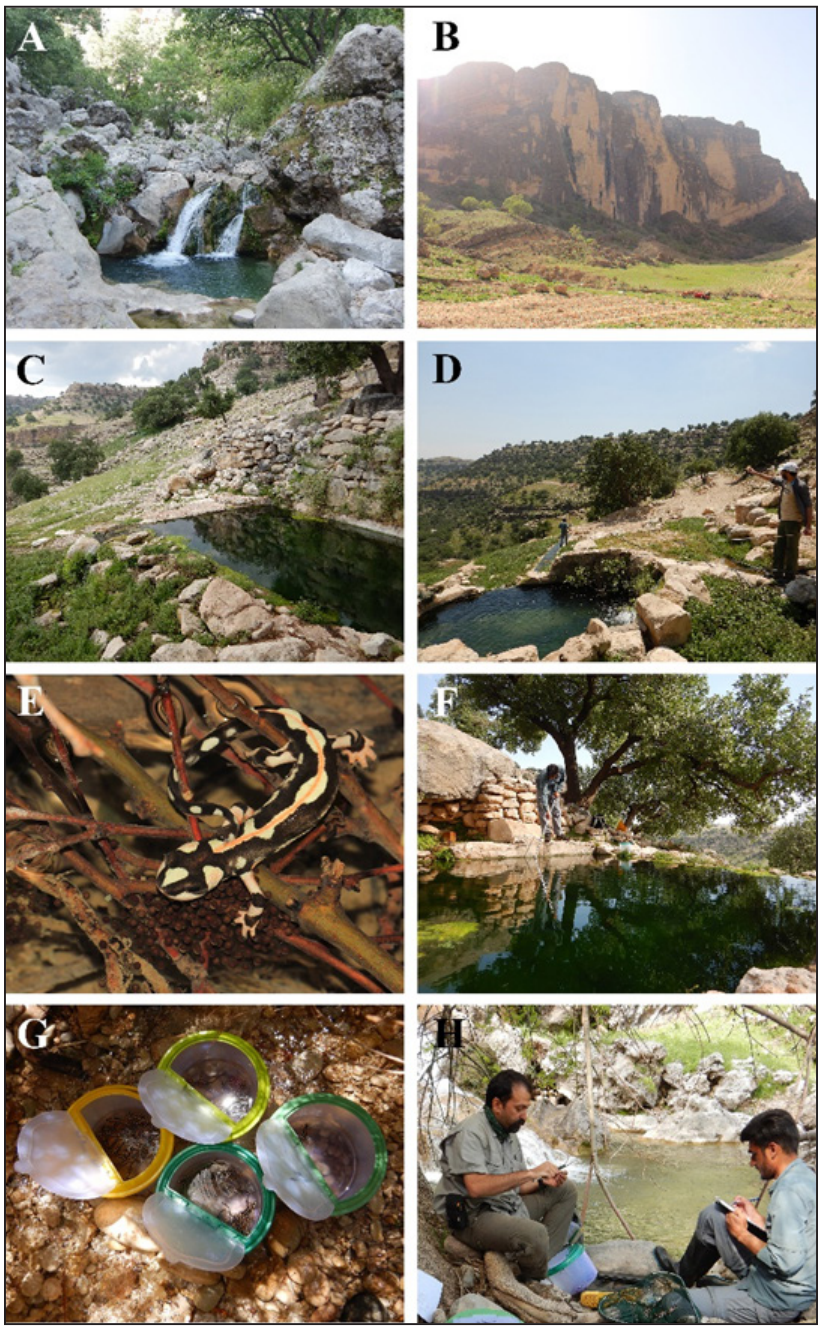

Figure 1. Habitats of Kaiser's Mountain Newts (*Neurergus kaiseri*). (A) Bozorg-Ab; (B) Dej; (C) Monjir; and (D) Alan. (E) Female Kaiser's Mountain Newt and its eggs in the natural habitat. Use of (F) a dip-net for catching specimens, and (G) plastic buckets during short-term specimen storage. (H) Measuring specimens. (All photographed by Barbod Safaei-Mahroo except H by Ashkan Miri).

Species of Wild Fauna and Flora (CITES) I. Additional threats include over-exploitation of water resources, including anthropogenic withdrawals of spring water. Because of the exploitation of springs for water supplies by nomads and farmers, vital aquatic resources required by this species are being depleted. Furthermore, restricted distribution and conservation challenges of this species make estimating sizes of populations extremely important.

Pond-breeding amphibians are valuable elements of mountain habitats and provide several essential ecosystem functions (Homan et al. 2018), such as serving as crucial links in food webs (Davic and Welsh 2004). One of the first steps to understanding the dynamics and structure of wildlife populations is determining the size of a population (Seber 1982; Phillips et al. 2001), which is necessary for assessing conservation status and is fundamental to many ecological

and conservation issues (Robinson et al. 2022). Data obtained from studies on population ecology helps wildlife and conservation managers develop long-term conservation programs to ensure survival of a species. Determining if a species is declining is impossible without accurate records of both past and current population sizes (Phillips et al. 2001), and standardized methods and techniques and accurate estimates of population size are necessary for development of meaningful programs. Furthermore, monitoring vulnerable and endemic species populations is vital to detect population fluctuations, especially decreasing population trends, so that the cause of the decline can be determined, and a proper management solution can be implemented quickly (Schwartz et al. 2015). The selection of a monitoring strategy can affect the outcome of the population assessment (Ward et al. 2017). Furthermore, the results of different studies cannot be compared without standardized methods (Heyer et al. 1994).

Since 2021, the Division of Wildlife Protection and Management of the Department of Environment (DOE) of Iran announced that estimating reptile and amphibian populations is a priority for provincial studies on wildlife. Because the DOE does not provide a standard and reproducible population estimation method and monitoring technique, comparing, and analyzing the data obtained for a particular species, even across neighboring areas, is impossible. Moreover, efficient, accurate, and effective long-term management decisions for the conservation and survival of amphibian species will be hampered by the use of non-standardized monitoring approaches (Safaei-Mahroo and Ghaffari 2020).

Despite the growing threats to *N. kaiseri* and its habitats, there is little information on the habitat requirements, estimates of size of populations, or of trends in fluctuation of size of populations. In this study, we estimate the size of populations of Kaiser's Mountain Newts breeding in the dominant habitat types in the Zagros Mountains. We evaluate three methods commonly used to estimate population size by analysis of multiple capture-mark-recapture occasions (Lincoln Peterson, Schumacher-Schnabel, and Jolly-Seber). Our goal is to provide a baseline population estimate for the Department of Environment of Iran and also for experts, researchers, and rangers, that can be used to compare with future studies, and that can be applied to monitor the population of this species annually.

Materials And Methods

Study area.—Our study area included six permanent breeding habitats in the mountain forest ecosystem of the southwestern part of the Zagros Mountains range in northern Khuzestan Province of Iran. These habitats included three areas in the eastern portion of the known distribution of *N. kaiseri* (Shoo-va-Lander Protected Area in Andika County, Jeryek in Masjed Soleiman County, and Darreh-bisheh in Andika County) and three areas in the western part of the distribution (Dej in Sardasht district, Dezful County, and Bozorg-Ab and Barik-Ab in the Alvar-e-Garmsiri, Andimeshk County; Fig. 2). Breeding of *N. kaiseri* apparently occurs earlier in eastern habitats, where relatively lower elevations are associated

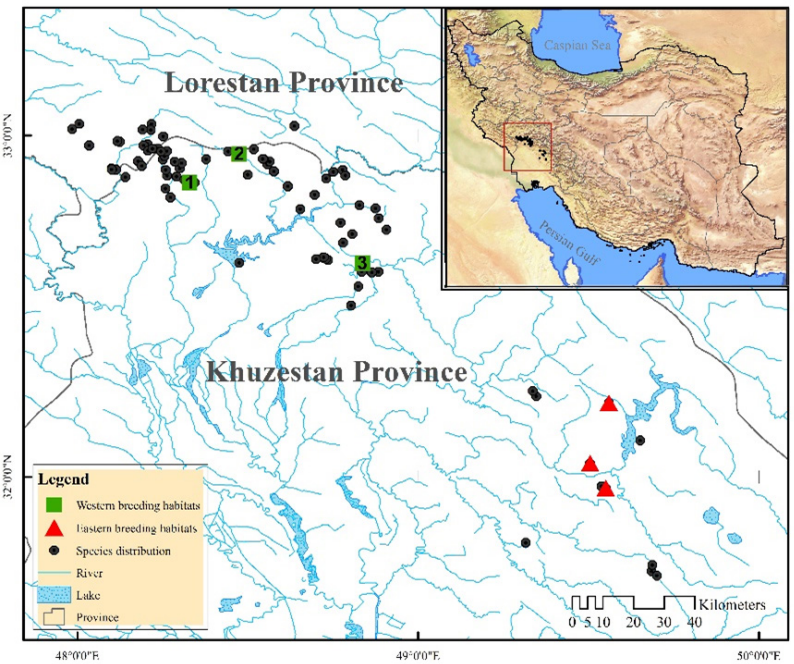

Figure 2. The global distribution of Kaiser's Mountain Newts (*Neurergus kaiseri*) and locations of study sites in the Zagros Mountains range of northern Khuzestan Province, Iran. Green squares represent study sites: (1) Barik-Ab, (2) Bozorg-Ab, and (3) Dej.

with higher temperatures, than in western habitats. When we searched breeding sites in the eastern habitats, we found only larvae (eggs had all hatched). Thus, we focused our estimates of population size in the western habitats. These three western habitats were separated by < 20 km (air distances), varied in elevation, vegetation types and structure, and water discharge. Based on discharge of selected stream habitats, we categorized the breeding sites into one of three groups: permanent (Bozorg-Ab); intermittent (Dej); and ephemeral (Barik-Ab, which includes Alan and Monjir). Despite these differences, all were permanent ponds, and all contained evidence of breeding by *N. kaiseri*.

Bozorg-Ab habitat (32°56'41"N, 48°28'26"E, elevation 1,250–1,335 m) is a relatively pristine area. This study site is characterized by free-flowing mountain streams in dense forests of *Quercus brantii* and *Pistacia atlantica*. Dej habitat (32°37'33"N, 48°50'10"E, elevation 840–875 m) in Haft Tanan No Hunting Area consists of mountain brooks dominated by *Quercus brantii* and Common Myrtle shrubs (*Myrtus communis*). To protect Myrtle shrubs, Natural Resources and Watershed Management Organization enclosed the entire habitat with fencing to restrict livestock. Monjir spring (32°51'58"N, 48°20'08"E, elevation 998 m) and Alan spring (32°51'33"N, 48°19'32"E, elevation 961 m) in the Barik-Ab habitat are water sources that have changed forms by the anthropogenic water withdrawals for use by domestic animals. The Monjir spring is a human-made small pool with a maximum depth of 3 m and a lower discharge level than Alan spring, which has been converted into long, shallow drinking troughs for domestic animals (Fig. 1).

Field methods.—We estimated the number of adult Kaiser's Mountain Newts breeding (size of the adult population). We performed capture-mark-recapture surveys using an exhaustive search during the peak egg-laying period, from 26 April to 7 May 2019, between 0900 to 1200. For the survey in stream habitats, we divided the stream length into 100 m sections (Price et al. 2012), including four sections in Bozorg-Ab and three sections at Dej, and we spent three person-hours capturing newts at each section. At Barik-Ab, we considered each spring (e.g., including Alan and Mojir) as separate sections and searched each for three person-hours. We used the same personnel and procedures during the recapture surveys to reduce human error and simplify the operation. We marked each newt with a toe-clip technique (Heyer et al. 1994), weighed, determined sex, and marked newts, and then we released them in the same spot as they were captured. We carried out three sampling sessions, separated by a 4-d interval; thus, surveys at each site were completed in 9 d. We collected newts using long-handled dip-nets (2-m, 50-cm triangle frame) in shallow parts of brooks and ponds. We captured newts by hand in shallow water, where the dip-nets could not be used. We recorded weight using a notebook digital scale (Black Leaf, Hennef, Germany; 2,000 g) to the nearest 0.1 g. We determined the sex of the newts using secondary sexual characters (Safaei-Mahroo 2014). We measured snout-vent length (SVL) and tail length (TaL) of < 20 newts at each site. We kept newts briefly in plastic buckets (30 cm diameter, 30 cm height), which we filled with the water taken from the point of capture, and we labeled the lids of each container with a waterproof marker to re-release marked individuals at their capture location. For each sampling session and site, we also calculated the sex ratio.

Data analysis.—We evaluated Bozorg-Ab and Dej habitats as linear features, and evaluated Monjir and Alan springs as area bases. We analyzed only the population size of adult newts and assumed that they did not leave the area during the 15-d sampling period. We tested the effect of marking and permanence of marks in the field. We assumed the chances of catching an animal were the same for all newts and that individuals do not lose marks (O'Donnell et al. 2008). We assumed a closed population in all of the models. We used Lincoln Peterson, Schumacher-Schnabel, and Jolly-Seber methods to estimate the population size of *N. kaiseri* and compared these methods using Ecological Methodology Software (Exeter Software, Setauket, New York, USA). Among these methods, we selected the most appropriate method for estimating population size. We also analyzed capture-recapture data with the program CAPTURE implemented in program MARK (Version 9.0), using the goodness-of-fit method to select the best model for estimating population size in the MARK software. CAPTURE Program in Mark software includes various models to estimate population size; we used four models, including M_T (Chao), M_T (Darroch), M_0, and M_B models (Table 1). We calculated 95% confidence limits for estimated populations at each site. We used Chi-Square test with a Yates correction to analyze the sex ratios (significant at the 0.05 level).

Table 1. Models in the Program CAPTURE (Otis et al. 1978) used to estimate population size of Kaiser's Mountain Newts (*Neurergus kaiseri*) occupying different habitat in the mountain forests of southwestern Iran.

Model	Description
M_0	The null model: The capture occurs entirely at random for all individuals, and all individuals have an equal chance of getting caught at any time.
M_T (Chao) M_T (Darroch)	The time model: Capture probability varies with the time of capture.
M_B	The behaviour model: After being caught once, individuals may change their behavior.

Results

We captured 1,765 newts (including recaptures), including 650 males and 1,115 females, during the field surveys in 2019. Female:Male (F:M) sex ratios varied among sites from 1.1–3.2:1 (Table 2). The total sex ratio was significantly female-biased ($X^2 = 9.34$, $P = 0.024$, n = 1,765); however, the sex ratio did not differ significantly at particular sites (all $P > 0.05$). Although not significantly different than 1:1, The Dej habitat had the lowest F:M sex ratio. Initially, we assumed the breeding season started earlier in the Dej than in other sites because of its lower elevation and warmer temperatures compared to other sites, but this habitat has a relatively balanced sex ratio. Sex ratios of 3.2:1, the highest female:male ratio, were observed during the second trapping occasion in the Monjir habitat, but this also was not significant (Table 2). We measured 156 newts; the largest was a gravid female with a SVL of 76 mm, a TaL of 83 mm, and a mass of 9.9 g. In comparison, the largest male newt had a SVL of 52 mm, a TaL of 73 mm, and a mass of 5.3 g.

Population size.—Estimates of populations sizes differed among localities based on the estimation model used (Table 3). The Lincoln-Peterson method was based on only two samples, but we compared estimates obtained from three sampling protocols: (1) a 4-d interval between the first (initial) capture and the second attempt (capture); (2) a 4-d interval between the second capture and the third attempt (capture); and (3) a 9-d interval between the first capture and the third attempt. The assumptions of the Lincoln-Petterson method are similar to those of the Schumacher-Schnabel method. We sampled each site three times. In comparison with the previous two methods, population size was smaller when using the Jolly-Seber method.

Population estimation in selected sites.—The Mb model was the best model ($M_0 = 0.14$, $M_t = 0.29$, $M_b = 0.58$) for estimating population size in the Bozorg-Ab habitat, with 1,403 individuals being estimated for this habitat. The M_0 model was the best-selected model ($M_0 = 1.00$, $M_t = 0.76$, $M_b = 0.00$) for estimating the population size of *N. kaiseri* in the Dej habitat, which estimated the population to be 491 individuals. The M_0 model also was the best fit ($M_0 = 1.00$, $M_t = 0.00$, $M_b = 0.35$) for estimating the size of the population in the Allan habitat, which was estimated to be 63 individuals. For the Monjir habitat, the M_0 model was best ($M_0 = 0.82$, $M_t = 0.73$, $M_b = 0.00$) for estimating the population size, which was estimated to be 321 individuals (Table 4).

Table 2. Sex ratios of Kaiser's Mountain Newts (*Neurergus kaiseri*) during trapping occasions of breeding populations occupying different habitats in the mountain forests of southwestern Iran. Abbreviations are F = number of females, M = number of males, Sr = Sex ratio (female:male), and X^2 = chi-square value for sexes.

Sampling sessions	Sex	Bozorg-Ab	Dej	Monjir	Alan
1	F	367	26	45	11
	M	210	21	27	4
	Sr	1.7:1	1.2:1	1.6:1	2.7:1
2	F	268	34	45	7
	M	171	31	14	5
	Sr	1.5:1	1.1:1	3.2:1	1.4:1
3	F	232	30	40	10
	M	129	21	13	4
	Sr	1.9:1	1.4:1	3.1:1	2.5:1
Total	F	867	90	130	28
	M	510	73	54	13
	Sr	1.7:1	1.2:1	2.4:1	2.1:1
	X^2	1.05	0.49	4.56	0.78

Discussion

Locally and globally, species loss begins with declining populations (Cruickshank et al. 2021). Thus, conservation of species requires reliable population estimates (Grimm et al. 2014), particularly for vulnerable and endemic species. Initial estimates of population sizes provide a baseline necessary for comparison future estimates to determine population trends, but trends in population size can only be determined with accurate estimates (Phillips et al. 2001). Furthermore, understanding the natural history of a species is necessary for accurate estimates of population size. For example, the breeding season is relatively brief in *Neurergus kaiseri* (Safaei-Mahroo and Ghaffari 2020), but some adults leave the breeding site before the season is finished (Schoorl and Zuiderwijk 1981; Tarkhnishvili 1986). Thus, only a fraction of the breeding population might inhabit a breeding site during peak reproductive activity, and estimates obtained from simple counts underestimate population sizes (Jehle et al. 2011). For example, based on direct counts of adult, juvenile, and larvae *N. kaiseri* at 12 sites, Sharifi et al. (2013) reported 650 individuals at Bozorg-Ab and 67 individuals at Barik-Ab. Mark-recapture methods allow for a more accurate estimate of population sizes (Jehle et al. 2011; Warwick 2021) and are commonly used to estimate sizes of populations newt in breeding ponds (Weddeling et al. 2004). Our estimates of 1,403 reproductively active *N. kaiseri* in the Bozorg-Ab habitat, 384 in the Barik-Ab [=Hajibarikab] habitat (63 in Alan spring and 321 in Monjir spring) indicate larger, and presumably more accurate, sizes of populations than obtained from direct or simple counts of individuals.

The estimated sizes of populations we obtained with the Lincoln-Peterson method with two sampling sessions and a time interval of 4 d are > 10 to 20% larger than

Table 3. Model estimates, numbers of individual Kaiser's Mountain Newts (*Neurergus kaiseri*) captured and recaptured, estimated population size, and confidence intervals using the Lincoln-Petersen, Schumacher, Schnabel, and Jolly-Seber estimators. Abbreviations are Nc-r = number of captured and recaptured newts, Rp = recapture probability, Pe = population estimate, 95% CI = 95% confidence intervals.

Estimator		Bozorg-Ab	Dej	Monjir	Alan
Lincoln-Petersen: S1	Nc-r	577	47	73	4
	Rp	0.20	0.19	0.19	0.20
	Pe	2,094	310	292	
	95% CI	1,856–2,438	190–719	199–535	
Lincoln-Petersen: S2	Nc-r	361	65	59	5
	Rp	0.20	0.10	0.13	0.20
	Pe	2091	244	354	
	95% CI	2,183–3,813	247–1,140	190–845	
Lincoln-Petersen: S3	Nc-r	439	51	53	4
	Rp	0.17	0.04	0.15	0.10
	Pe	2748	442	328	
	95% CI	2,298–3,508	133–727	214–673	
Schumacher	Nc-r	1377	163	185	41
	Rp	0.19	0.11	0.18	0.18
	Pe	2117	507	322	65
	95% CI	2,071–2,282	119–227	230–536	39–182
Schnabel	Nc-r	1377	163	185	41
	Rp	0.19	0.11	0.18	0.18
	Pe	2173	461	320	64
	95% CI	1,719–2,952	299–743	233–460	34–157
Jolly-Seber	Nc-r	1377	163	185	41
	Rp	0.19	0.11	0.18	0.18
	Pe	2069	168	349	20
	95% CI	1,669–2,887	85–674	189–944	13–48

the other two methods that used three capture and recapture surveys. The Lincoln-Peterson method that used 9 d between two sampling sessions, estimated the population size of *N. kaiseri* 30 to 70% larger than Schumacher-Schnabel and Jolly-Seber, which used three capture and re-capture sessions. Our data for the 9-d time interval for the Lincoln-Peterson S3 estimator model indicates the population size of *N. kaiseri* is one-third that of other population methods used. Also, the probability of re-capturing has been reduced to 4%. We recommend that if the Lincoln-Peterson method is used, based on the behavioral characteristics of *Neurergus* at the time of reproduction especially for males that leave the reproduction site sooner, the time intervals of 4 d between the two captures is used to meet the assumption of a closed population.

The higher the number of recaptures, the greater the accuracy of the estimate of size of a population. Nevertheless, the Schumacher-Schnabel method assumes a closed population, a condition likely violated in our study; consequently, estimates based on this method are suspect. Furthermore, the Schumacher-Schnabel method is not recommended due to the behavioral characteristics of the species during reproduction season, especially males, which leave the reproduction location more quickly. *Neurergus kaiseri* should be re-captured every two days to obtain a more appropriate result using the Schumacher-Schnabel method; however, the marked individuals do not have enough time to mix uniformly with the unmarked individuals in the population and cause unequal catchability if the interval between capture and re-capture is only 2 d. Therefore, we do not recommend estimating population sizes of *N. kaiseri* using the Schumacher-Schnabel method.

Several studies have estimated newt and salamander populations using Jolly-Seber (Bell 1979; Bendik 2017; Warwick 2021), which assumes an open population, which is more realistic for pond breeding salamanders. Based on the behavioral characteristics of the species at the time of reproduction in this study, the population should be considered an open population. During our study, we realized that, compared to females, male newts spent less time in ponds and aquatic environments during the breeding season. Furthermore, the Lincoln-Peterson

Table 4. Program MARK population estimates of Kaiser's Mountain Newts (*Neurergus kaiseri*). Abbreviations are Nc-r = Number of captured and recaptured newts, Rp = Recapture probability, Pe = Population estimate, Se = Standard error, CI = 95% confidence intervals.

Estimator		Bozorg-Ab	Dej	Monjir	Alan
Jolly-Seber: M_0	Nc-r	1111	145	152	33
	Rp	0.2	0.1	0.1	0.2
	Pe	2214	491	321	63
	Se	99.77	101.64	41.95	15.78
	95% CI	2,036–2,428	343–753	257–425	45–112
Jolly-Seber: M_b	Nc-r	1111	145	152	33
	Rp	0.4	0.2	0.3	0.2
	Pe	1403	271	217	50
	Se	47.60	15.99	30.5	18.86
	95% CI	1,324–1,512	244–306	180–307	37–130
Jolly-Seber: M_t	Nc-r	1111	145	152	33
	Rp	0.2	0.1	0.1	0.2
	Pe	2172	486	319	63
	Se	96.61	99.73	41.17	15.14
	95% CI	1,999–2,378	340–743	256–420	45–109

and Schumacher-Schnabel methods do not meet the assumption of population closure; therefore, the Jolly-Seber method provides a more appropriate estimate by assuming an open population in the studied sites. Our estimates of the sizes of populations of *N. kaiseri* using the Jolly-Seber method (e.g., 1,403 individuals in the Bozorg- Ab habitat, 491 individuals in the Dej habitat, 63 individuals in the Alan habitat, and 321 individuals in the habitat of Monjir) are presumably more accurate than those estimates using other methods. Thus, we recommend using the Jolly-Seber method to estimate population size of *N. kaiseri*, that sampling bouts be separated by 4 d, and that at least three sampling bouts are used. Furthermore, we suggest that the best time to estimate the population size of this species in southern Zagros is from 15 April to 15 May (the breeding season). Because accurate information about the time of reproduction throughout the range and different habitats of this species is unavailable, we suggest that habitat characteristics (e.g., canopy, discharge, water depth, and topographic features including slope percentage, slope direction, and height) should be used to determine when to begin estimating population sizes. Because of the lower elevations of the eastern habitats of Khuzestan Province, breeding begins about one month earlier than in the western habitats. Also, habitats with low water discharge have shorter reproduction periods because of higher water temperatures. Accordingly, prior to conducting field surveys to estimate population sizes of *N. kaiseri*, we recommend conducting preliminary and pilot studies.

Habitat fragmentation and illegal trade are the two main threats to this species (Safaei-Mahroo 2014). The species has been listed on Appendix I of the CITES due to high purchasing demand (CITES 2010). Because of increased pressure on populations, the DOE of Iran has increased penalties on harvesting *N. kaiseri* for the pet trade. Regarding conservation status, *N. kaiseri* was listed as Critically Endangered on the IUCN Red List in 2006 but was downlisted to Vulnerable in 2016 (IUCN 2016). The change in conservation status from Critically Endangered to Vulnerable was based on an increase in the overall distribution area (minimum convex polygon method) of 8.948 km^2 (IUCN 2016), without considering the habitat requirements and characteristics of the species. In the last decade, several small populations have been found in disjunct isolated habitats (Safaei-Mahroo and Ghaffari 2020), but neither distribution nor overall sizes of populations have increased significantly. The extent of occurrence is just 4,961 km^2 (Safaei-Mahroo et al. 2023). Because of low vagility, *N. kaiseri* is restricted to habitats adjacent to springs and mountain streams in isolated and fragmented habitats. Illegal harvest and increasing pressure on all habitats are threatening the survival of known populations; consequently, we recommend that the conservation status of the species should be reconsidered.

Implications for a national monitoring plan.— Population estimates are an important first step to developing a national conservation plan, but quantifying habitat associations (Suzuki et al. 2008), quantifying climate and other threat sensitivities (Muths et al. 2017)

Table 5. Recommended guidelines and techniques for estimating population and monitoring. mountain newt populations (*Neurergus* spp.) in Iran.

Steps	Description
Permissions	Necessary permits must be obtained from appropriate organizations or agencies such as the Department of Environment.
Define the target population	Adult newts at the breeding site during the breeding season.
Scales of survey	Small geographic area
Aquatic survey area	Mountain breeding streams, brooks, and springs within species distribution ranges.
Selection of study sites	To select study sites, at least two habitat visits are required.
Seasons to conduct field surveys	During the peak of breeding season (from April to May for *Neurergus kaiseri*).
Time for surveys	During the day, between 0900 and 1200.
Estimating method	Capture-mark-recapture
Survey method	Active searching, and visual encounter survey.
Equipment	Survey forms, clipboard, GPS, rubber boots or waders, measuring tape 50m, plastic buckets, long-handled dip-nets, nail clippers or small scissors.
Biometry equipment	Data sheets, camera, digital scale, digital caliper,
Marking method	Toe-clipping
Survey Protocol	Same procedures and observers with sufficient training and experience.
Number of fieldworkers	3–4 experienced investigators
Person-hours	Three person-hours for each site (100 m sections)
Sampling interval	4 d interval
Sampling sessions	Population is recommended to be sampled at least three times
Study length	The study length is recommended to be short.
Population estimator	Jolly-Seber method.
Identification of amphibian references	Safaei-Mahroo, and Ghaffari. 2020; Safaei-Mahroo, et al. 2023.

and assessing local and regional population trends (Hossack et al. 2013) are also critical factors that need to be considered. Our study was shared and presented to the DOE of Iran and the General Department of Environment of Khuzestan Province for consideration in the National Mountain Newts Population Estimation and Monitoring Plan (Safaei-Mahroo et al. 2020). Consequently, it was used as a guide for estimating and monitoring mountain newt populations in Iran. As requested by the Khuzestan and West Azerbaijan General Department of Environment, the results and estimates of the population method were presented to managers, environmental experts, and rangers there in 2021 and 2022. Our study also can provide guidelines for a Master's theses on populations of *Neurergus derjugini* (Karimi 2020; Karimi et al. 2021). We have provided specific guidelines and techniques for estimating population size and for monitoring mountain newts (*Neurergus*) that we think should be followed when studying this group of amphibians (Table 5).

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