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Incorporating habitat suitability and demographic data for developing a reintroduction plan for the critically endangered yellow spotted mountain newt, *Neurergus derjugini*

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In reintroduction programmes for amphibians, data on age structure in hosting populations and choices of life stage or age groups in releasing captive bred individuals are often missing. Similarly, employing site selection procedures for selecting appropriate reintroduction locations are often neglected. Here, we obtained data on longevity, age at maturation, and age structure from skeletochronological data in a free living population of the yellow spotted mountain newt, *Neurergus derjugini*. A maximum longevity of 13 years for males and 12 years for females showed that *N. derjugini* is a long living newt with a stable age structure. We also employed maximum entropy modelling, geographic information system, and multi-criteria decision analysis to obtain ranked suitability scores for reintroduction sites. Finally, we determined post-release survival rates for different life stage and age groups of *N. derjugini* including 30 eggs and 60 individuals of six-months old larvae, one and three-year old juveniles, and six-year old adults (15 each) born and raised in a captive-breeding facility and released into mesh enclosures in a selected stream. Over 10 visits to the site before and after overwintering, the survival rates for eggs, larvae, one and three-year juveniles and six-year old adults were 25, 80, 86.66, 93.33 and 53.33 % respectively. Applying survival rates obtained from current experimental reintroductions through a static life table suggest that an optimal release strategy to arrive at a numerical target of 100 adults aged three can be achieved by reintroduction of 650 fertilised eggs and fostering them in meshed enclosures in the selected stream.

Keywords: reintroduction, life table, GIS, survival rate, critically endangered species, *Neurergus derjugini*

INTRODUCTION

Captive breeding and reintroduction programmes are important conservation tools that are playing an expanding role in preserving endangered species. The number of captive breeding plans has increased rapidly in recent years. Harding et al. (2016) reviewed captive breeding programmes involving 213 amphibian species and showed a growing number of parameters resulted from life in captivity have an influence on a variety of characters of the amphibian species. These authors have reported an increase of 57 % in the number of amphibian species involved in conservation breeding and reintroduction programmes since 2007. Biega et al. (2017) reported 532 amphibian species (7 % of all amphibian species) held ex-situ, compared to 4 %, reported five years earlier. Moreover, research focusing on the reintroduction of captive-bred individuals to the natural environment concerns several important issues such as adjusting to the new environments, health control of released individuals, genetic management, and long-term monitoring of released animals (Armstrong & Seddon, 2008).

Despite the growing number of captive breeding and reintroduction programmes operated for conservation purposes to rescue a number of endangered species, the consequences of these programmes have not always been satisfactory (Hedrick & Fredrickson, 2008). There are many reports demonstrating that these programmes have encountered various shortcomings. In amphibians, some captive breeding programmes have experienced poor nutrition (Pough, 2007), diminished natural behaviours (Burghardt, 2013), inactivity in the natural environment (Keulen & Janssens, 2017), inability to recognise natural foods (Olfert et al.,1993), a variety of diseases due to nutritional deficiency (Densmore & Green, 2007) and chytridiomycosis infection (Parto et al., 2013), failed reproduction (Browne & Zippel, 2007), loss of social interactions (Rabin, 2003), changes in morphology of natural skin colouration (Ogilvy et al., 2012), loss of anti-predator response to predators (Kraaijeveld-Smit et al., 2006), loss of predation ability (Salehi & Sharifi, 2019), a reduced immune response (Keulen & Janssens, 2017) and loss of genetic diversity due to inbreeding (Zippel et al., 2011).

There are also cases of pragmatic failures in various efforts of breeding and release of threatened species that may cast doubts on the value of captive breeding, and as a consequence, reintroduction as a conservation procedure for endangered species (Griffiths & Pavajeau,

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2008). However, there are many situations in which captive breeding is the only conservation choice available (Stuart et al., 2004). Meanwhile, efforts to expand current understanding of ecology and behaviour of reintroduced species are growing (Pough, 2007).

Questions regarding the importance of captive breeding and subsequent reintroduction of threatened species will continue to appear as more studies are carried out on these programmes (Armstrong & Seddon, 2008). In the view of the practical irreversibility of many current threats to amphibians in their natural environments, captive breeding and reintroduction are becoming an essential conservation tool (Griffiths & Pavajeau, 2008; Harding et al., 2016).

Owing to reintroduction efforts, individuals born and raised in captivity can help to restore threatened wild populations or prevent rapidly diminishing populations. There are several factors attributed to causing failure of reintroduced individuals to survive in a new environment, including the source of the founder populations (Kleiman, 1989), the demography (number, sex ratio and age structure) of the release group (Maran et al., 2009), genetic diversity of the released individuals (Godefroid et al., 2011), choice of the life stage (Canessa et al., 2014; Sarrazin & Legendre, 2000), failure to remove previous threats (Moseby et al., 2014) and selection of inappropriate habitat for resource provision (Cheyne, 2006; Osborne & Seddon, 2012). Inadequate population sizes and restricted distribution range can greatly increase the risk of species extinction (Converse et al., 2013). Griffiths and Pavajeau (2008) investigated 58 reintroduction projects involving different species of amphibians and reported 22 projects and 13 were considered highly successful. In an assessment on the degree of success and failure of 58 reintroduction programmes reported in the sixth Global Reintroduction Perspectives by the IUCN (Soorae, 2018), 23 projects were considered highly successful, 24 were successful, 17 were partially successful and 4 were listed as failures.

Our main goals in the present study are to (1) determine longevity, age at maturation, age structure for *N. derjugini* based on skeletochronological data, to (2) integrate species distribution modeling (MaxEnt), geographic information system (GIS) and multi criteria data analyses (MCDA) methodologies for selecting a suitable reintroduction site, and to (3) determine survival rates of various age class *N. derjugini* including eggs, larvae with six-months old, juveniles at one-year and three-years-old, and adults at six-years-old, within a mesh enclosure to determine optimum choice of age groups and life stage for a reintroduction programme.

MATERIALS AND METHODS

The species

The yellow spotted mountain newt, (*Neuregus derjugini* formerly known as *N. microspilotus*), is listed as Critically Endangered by the IUCN because of its very small area of occupancy in its breeding streams (<10 km^2), fragmented habitats, continuing decline in the extent and quality of aquatic habitats, habitat degradation, drought, and pet trade (Sharifi et al., 2009). Recent field studies on distribution and abundance (Afroosheh et al., 2016), activity pattern and home range (Sharifi & Afroosheh, 2014) and also laboratory experiment on growth and development (Vaissi et al., 2018; Vaissi & Sharifi, 2016a, 2016b), and captive breeding (Sharifi & Vaissi, 2014; Vaissi & Sharifi, 2018) shows that *N. derjugini* is a long-living mountain newt that reproduces in highland streams at low density and feeds on benthic macroinvertebrate communities in cold and clean water in highland first-order streams in the mid-Zagros range. The maximum linear distance between the most segregated breeding streams in the south and north parts of the species range is only 205 km. However, localities inhabited by *N. derjugini* are separated with nearest neighbour distances averaging 7.95 km. Surveys on the abundance of *N. derjugini* in 32 of the 42 localities within the Iranian range of *N. derjugini* resulted in the total visual count of 1,379 adults, juveniles, and larvae (mean/stream = 43; range, 1−601). Most of these observations (51 %) were found in just two of the localities, 44 % were found in 14 streams, and the remaining 5 % were scattered among 16 streams (Afroosheh et al., 2016).

Skeletochronology

The *N. derjugini* used in the skeletochronology study (37 males, 44 females) were all caught in the daytime on the 3rd June 2011, in Kavat Stream. The sex of each mature individual was determined according to Sharifi et al., (2012) as males having a fleshy protuberance at the base of the tail, whereas females have a prominent cloaca. A toe clip (i.e., second or third) was cut and kept in 70 % ethanol. *Neurergus derjugini* collected for tissue sampling were kept in small (30 cm×30 cm) pools for about two hours to see if toe amputation caused any visible side effects such as bleeding. No bleeding was seen and the newts were released at their collection site. The phalange was fixed with 10 % buffered neutral formalin, dehydrated in a graded series of ethanol, cleared in xylene, embedded in paraffin wax, and sectioned serially at 8–10 µm with a rotary microtome. Ten slides were stained using Harris hematoxylin and observed with a light microscope (Leica, Galen III, Leica Microsystems, Wetzlar, Germany). Bone sections were photographed by a digital camera (Leica with Dinocapture 2). All photographs were taken at the same magnification, allowing for simultaneous comparison and facilitating the analysis of the bone growth pattern (Farasat & Sharifi, 2016). The age of newts was determined by counting the lines of arrested growth (LAGs).

Site selection for experimental reintroduction - Maximum entropy model (MaxEnt)

The site selection for the present experimental reintroduction of various age groups of the *N. derjugini* was initiated with a habitat suitability analysis based on a presence-only model (MaxEnt) to provide potential distribution for this species in Iran and Iraq (Sharifi et al., 2017). Within predicted habitats with suitability score of 0-100 %, the site selection operated using a GIS-MCDA decision analysis.

- Geographic Information System (GIS)
Spatial data for complete GIS analysis in the highlands of the mid-Zagros mountains, rural, and regional planning are prepared by various organisations. Sources and attributes of geospatial information used in the GIS process to select suitable areas for the reintroduction are shown in Table 1. GIS data obtained from different sources was used to perform spatial analysis via ESRI ArcGIS 10.2 software.

Table 1. Source and attributes of geospatial information used in the GIS process to select suitable areas for the *N. derjugini* area in western Iran and eastern Iraq. USGS: United States Geological Survey; IFRWMO: Iranian Forests, Range and Watershed Management Organization; IMO: Iran Meteorological Organization; SCI: Statistical Centre of Iran.

No	Data type	Data sources	Resolution
1	Digital elevation model (DEM)	USGS	10 m
2	Human settlement and village	SCI	1: 250000
3	Main road	IFRWMO	1: 250000
4	Floodplain, lake, dam reservoirs	IMO	1: 250000
5	Land use	IFRWMO	30 m
6	MaxEnt modeling output	Sharifi et al. (2017)	-
7	Newt localities	Afroosheh et al. (2016)	-

In the current study, 15 spatial attributes are involved in the site selection (Table 2 and 3). These attributes are distinguished as exclusionary (Table 2) and non-exclusionary (Table 3) criteria. In this study, exclusionary criteria are assumed as hazardous to the site selection and therefore are considered as decisive factors, which have been employed to keep away from the reintroduction site by assigning various buffers to these areas. These include stream density, connectivity of the localities reported for the newt, vegetation cover, land use, human settlements, village density, main roads and highways, flood plain, lake and dam reservoirs. Exclusionary criteria and their buffering values to identify reintroduction site are present in Table 2.

The second group of spatial data used to select reintroduction site for the yellow spotted mountain newt comprises non-exclusionary criteria relevant to environmental parameters and field observations. The non-exclusionary criteria used in this study include conservation integrity, suitable habitat based MaxEnt score (0-100), number of newt localities, stream density, distance to the protected area, villages density, distance to the main road. Exclusionary and non-exclusionary criteria are not necessary mutually exclusive. For example, although the village's density or main road is set as exclusionary criteria within the reintroduction site distance to the villages density or main road can act as a non-exclusionary criterion.

- Multi Criteria Decision Analysis (MCDA)
Here, multiple criteria decision analysis is considered as a family of techniques that aid us to compare and evaluate the alternatives of land for development of a conservation-oriented goal. This technique has been used for about two decades with geographic information systems (GIS) to analyse spatial problems.

In the process of aggregation of all criteria, weights were assigned according to how important each factor was considered, as indicated in Table 3. The implemented technique of factor pair-wise comparison was used as defined by Saaty (1987) in the context of a decision-making process known as the analytic hierarchy process (AHP) (Saaty, 1987; Pavlikakis & Tsihrintzis, 2003). Also, for all non-inclusionary criteria rating curves were developed according to Sharifi et al. (2009) as indicated in Table 4. The overall suitability for a reintroduction site was obtained according to the following equation:

$$SI = \sum_{i=1}^{n} (WiXi)$$

where suitability index (SI) designates the suitability score for reintroduction site attained by *n*th alternative; the Wi is the weight of the factor calculated by using the

Table 2. Exclusionary criteria and their buffering values used for the selection the suitable areas for reintroduction.

No	Criterion	Buffering
1	Stream density	Exclude stream density less than 0- 0.007 Km^2
2	Connectivity: ridge density	Exclude areas over 2200 m.a.s.l
3	Vegetation cover	Exclude unsuitable terrestrial vegetation cover
4	Land use	Excluded all arable lands
5	Human settlement	
	City between 1000-10000 population	1000 m buffer zone
	City between 10000-100000 population	2500 m buffer zone
	City between 100000-500000 population	5000 m buffer zone
6	Village density	Exclude areas with high village density (more than 3.68 Km^2)
7	Main roads and highway	1000 m buffer zone
8	Floodplain, lake and dam reservoirs	1000 m buffer zone

Table 3. The relative importance of non-exclusionary criteria obtained based on the nine-point rating system. These values obtained based on a pair-wise comparison of all criteria using the weighted linear combination described by Saaty (1987). To rate each pair-wise comparison and to fill in the matrix cells, the relative importance of the row variable to its corresponding column variable is considered.

No	Criteria	Weights
1	Conservation integrity	0.25
2	Habitat suitability score	0.20
3	Number of newt localities	0.16
4	Stream density	0.13
5	Distance to protected area	0.11
6	Villages density	0.09
7	Distance to main roads	0.06

pair-wise comparison between various criteria (Table 3); and Xi is a suitability value obtained from rating curves (Table 4).

- Field studies

Following overlaying of the exclusionary layers, a series of field studies were conducted in order to provide more information for a suitability evaluation. Also, extensive use of Google Earth Pro and Ultimate Maps Downloader was employed in order to collect information about spatial data regarding land use alteration, development projects, and changes in vegetation cover that were not represented in the exclusionary spatial layers. Within patches of suitable areas obtained from overlaying of various layers, eight plots of land were selected by removing marginal areas to provide more interconnected area.

Finally, the suitability of different potential reintroduction sites in the patches with highest suitability values (15 water bodies) were assessed against several criteria including:

1- Habitat parameter including: altitude (m); position (l/s); food availability.

2- Water quality including: pH, salinity (EC; μS/cm), turbidity (NTU), dissolved oxygen (mg/L), water temperature (^{0}C).

3- Structural parameters including: permanence of water body and predator (snake, crab, buffo).

These data were not used quantitatively in the process of evaluating of the suitability index, but were used to represent, qualitatively, various characteristics of the breading streams.

Trial reintroduction

The captive breeding facility (CBF) at Razi University, Kermanshah Iran, established in 2010, when the Mohamed bin Zayed Species Conservation Fund helped to develop and implement a conservation management plan for the yellow spotted mountain newt. Details of the CBF and a trail reintroduction of the juvenile born in the CBF are explained in Sharifi and Vaissi (2014) and Vaissi and Sharifi (2018). All eggs, larvae, juveniles and adults used in the present study had been maintained in the CBF for all of their life and were siblings of individuals from a known locality. The larvae and adult newts chosen for the experimental reintroduction were arranged for a visual health inspection and behavioural examination (absence of skin slough and wound, viability and sensitivity to stimulus) to ensure they were healthy (Vaissi & Sharifi, 2018). In June to September 2017 across three occasions, 30 eggs, 15 six-months old larvae, 15 one-year old, 15 three-year old, and 15 six-years old adults were released inside 10 meshed enclosures (size of the mesh bag: length: 60 cm; width: 50 cm; height: 15 cm) in the Shelmav stream (N35^{0}00'56.5"; E046^{0}26'41.4") in Khaneghah villages in Paveh, Kermanshah province in Iran.

Releases into mesh bags were as follows; on the first occasion, for each age class (one, three, six-years old), except six-months old larvae, seven newts (total N= 21) and 15 eggs; on the second occasion, for each age class, except six-months old larvae, eight newts (total, N= 24) and 15 eggs; and on the third occasion, six-month old larvae (total, N= 15) were released. In addition, for five bags, in each bag, seven newts, while for five other bags, for each bag, eight newts were released. Each mesh bag was made of loose plastic with 5×5 mm grids allowing the flow of water and food in the stream. The grid was smaller (2×2 mm) for bags containing eggs. In order to standardise conditions inside the mesh bags and prevent direct contact of the newts, several stones and moss were placed inside the bags. On every visit, the stones and moss were replaced. Although we regularly provided mealworm as additional food, the flow of water through the mesh bags provided plenty of food as in each visit, we found food in the bags, including earthworms, red worms, gammarus and larvae of insect in the meshed bags and between moss. However, to ensure enough food, five mealworms (*Tenebrio molitor*) per newt, were added to the mesh bags.

The reintroduction site and mesh bags were monitored on 10 occasions: 2 June 2017; 16 June 2017; 30 June 2017; 21 July 2017; 6 August 2017; 1 September 2017; 27 September 2017; 30 October 2017; 19 January 2018; 9 March 2018. For each monitoring, the newts within each mesh bag were counted and survival rate determined as a percentage of live remaining.

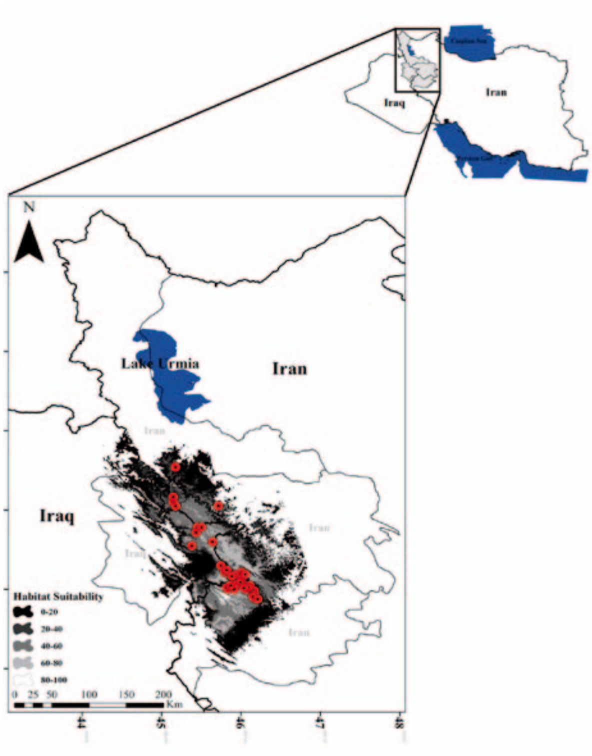

Figure 1. Habitat suitability map developed by Maximum entropy model (MaxEnt) for the *N. derjugini* based on known distribution (red-circle) in Kermanshah, Kurdistan and West Azarbaijan provinces in the western Iran and Sulaymaniyah province in eastern Iraq. Percentage probability of occurrence is pooled in five categories and expressed as different shades of grey shown in the legend.

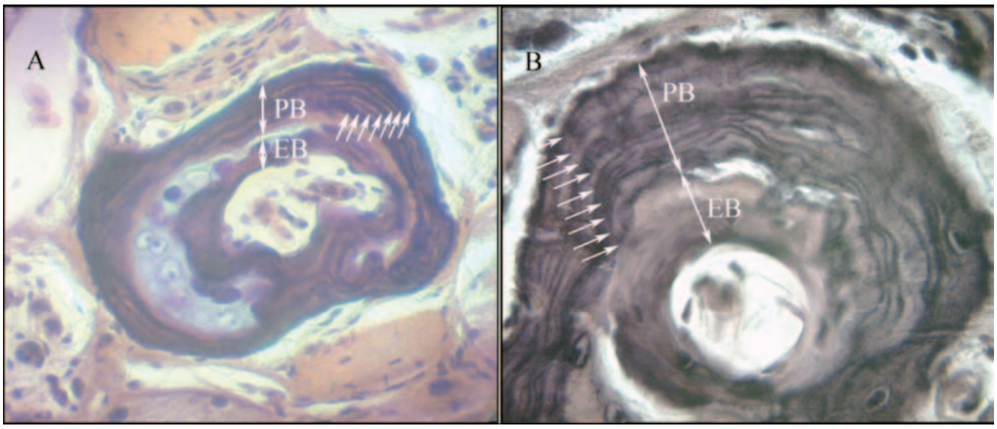

Figure 2. Transverse sections of phalange of *N. derjugini*, viewed with a light microscope, showing seven **(A)** and nine **(B)** lines of arrested growths LAGs (arrows). EB, endosteal bone; PB, periosteal bone.

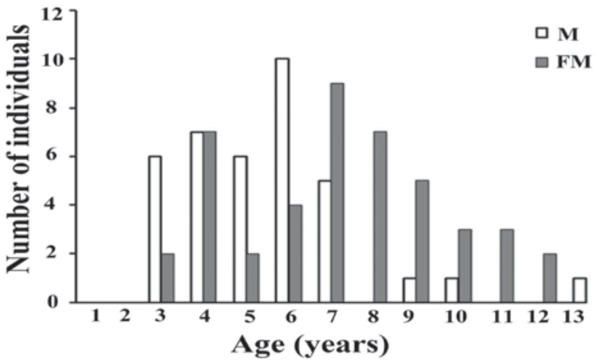

Figure 3. Frequency distribution of aged individuals of male and female *N. derjugini* sampled in Kavat stream.

RESULTS

The distribution of the yellow spotted mountain newt is limited to the conjunction of the western Iranian Plateau and the northern Mesopotamian Plain in western Iran and eastern Iraq. This area has a highly heterogeneous topography and climate. Within this area, the MaxEnt model identified substantial uninterrupted areas of geographic distribution (Fig. 1). In the remaining area, the model detected only very limited and scattered sites with low suitability. The model also detected some areas characterised by a high presence likelihood where records for the species are lacking (Fig. 1). This is particularly well pronounced in Iraqi territory at the middle of the distribution range. The model has shown a strong predictive ability, with the area under curve (AUC) of the ROC analysis providing a value of 0.92 (Sharifi et al., 2017).

Skeletochronology

Examples of sections prepared from toe clips of *N. derjugini* are illustrated in Figure 2. The age of each newt was determined by counting the lines of arrested growth in the compact bone layers in the outer and broader layer of periosteal bone (PB) and any line in the endosteal bone (PB) was neglected. Of the 81 newts over three-years old (larvae and post-metamorphs are excluded) for which toe clips were available for age estimation, 37 were male and 44 female. Adult females made up 54.32 % of the samples with a close sex ratio (male: female =1:1.21). Percentage of both male and female *N. derjugini* increased from age three to six. Males and females of ages three to six years constitute 61.72 % of the total sampled *N. derjugini*. A consistent decrease in the number of males and females *N. derjugini* is seen in age 7-13 (Fig. 3; Table 5). The average and standard error of longevity for 81 male and female *N. derjugini* are 6.44±0.27 years. The highest longevity reached was 13 years for males and 12 years for females. The minimum number of LAGs found in the mature newts shows that both male and female *N. derjugini* become sexually active at age three.

Site selection for experimental reintroduction
- Maximum entropy model (MaxEnt)

The potential distribution of *N. derjugini* in Iran and Iraq obtained based on a presence-only model (MaxEnt) model (Sharifi et al., 2017) is shown in Figure 1. All localities reported for *N. derjugini* are used to anticipated potential distribution of this species and in the site selection for reintroduction (Fig. 1, red circle).

- Geographic Information System (GIS)

In the basic map of the study area provided by the species distribution modelling (MaxEnt), the initial site selection for selecting reintroduction sites involved preparing the mask maps. Based on the exclusionary criteria, a map divides the study area into two suitable and unsuitable portions for *N. derjugini* illustrates in Figure 4. These mask maps include (1) exclude low connectivity among localities reported for *N. derjugini*, presence of high mountain georeferenced as areas with high ridge density (elevation>2200 m.a.s.l); (Fig. 4A), areas with unsuitable land use including (2) agricultural lands (Fig. 4B) and (3) terrestrial vegetation cover (Fig. 4C), (4) all main roads and highways (Fig. 4D), (5) inappropriate distance to human settlement (Fig. 4E), (6) high village density (Fig. 4F), (7) lake, dam reservoirs and floodplain area (Fig. 4G), and (8) low stream density (Fig. 4H). Overlaying of these mask maps resulted in several groups of suitable

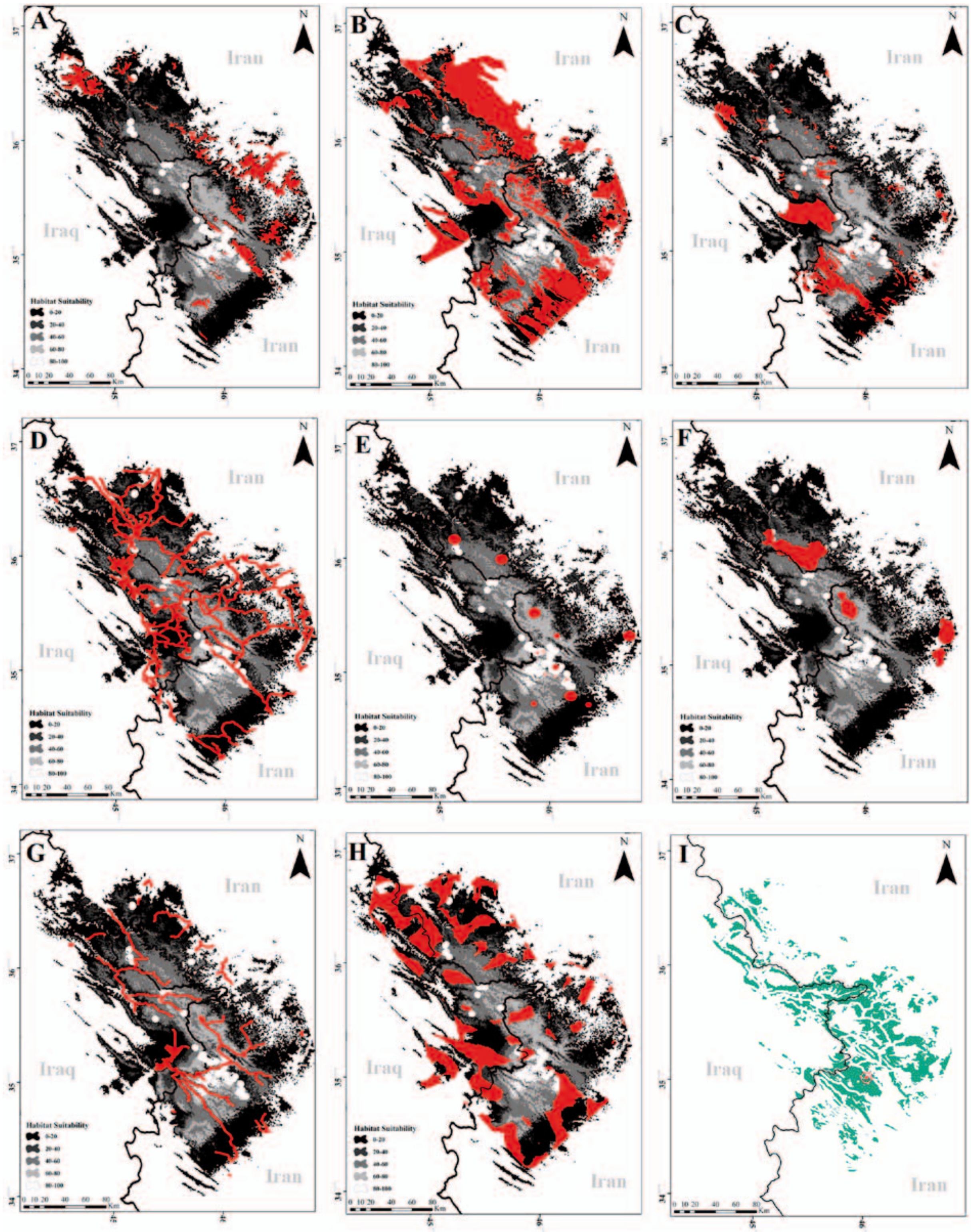

Figure 4. Mask map prepared within MaxEnt range to exclude **(A)** exclude low connectivity among localities reported for *N. derjugini*, presence of high mountain georeferenced as areas with high ridge density (elevation>2200 m.a.s.l), areas with unsuitable land use including **(B)** all agricultural lands and **(C)** exclude terrestrial vegetation cover, **(D)** all main roads and highways, **(E)** inappropriate distance to human settlement **(F)** village density, **(G)** lake, dam reservoirs and floodplain area, and **(H)** low stream density. **(I)** Suitable habitat (green path) from overlaying of these mask maps. The red circle in Figure 3I: reintroduction site (Shelmav stream; Khaneghah). The buffering values as indicated in Table 2.

locations shown in Fig. 4I.

Further field studies were conducted in order to provide more information for the suitability evaluation following the determination of the suitable sites by overlaying the eight spatial layers. In these field studies, several new criteria and characteristics such as the presence of natural boundaries for the nominated area, vigour and vitality of vegetation cover, edge effect resulted from the shape of the nominated reintroduction site, and landscape appeal has been considered under a single

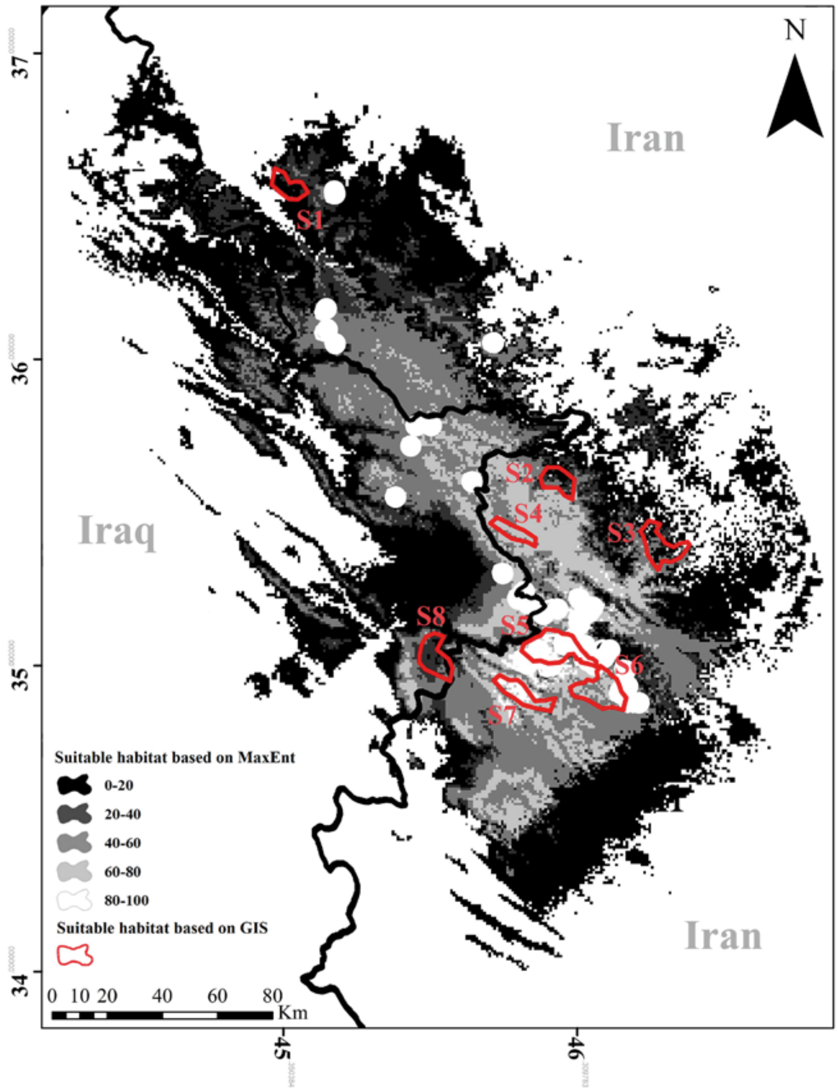

Figure 5. Eight most suitable sites for reintroduction obtained following overlaying exclusionary criteria, species distribution modeling (MaxEnt) and multi-criteria decision analyses (MCDA) of non-exclusionary criteria.

non-exclusionary criterion as "conservation integrity". The non-exclusionary criteria included in the second phase comprise conservation integrity; habitat suitability based on MaxEnt score, number of newt localities, stream density, distance to reintroduction site, village density, and distance to main roads.

- Multi Criteria Decision Analysis (MCDA)

All non-exclusionary criteria were assigned a weight calculated from pair-wise comparison of all criteria as described by Saaty (1987). Relative importance (weight) of seven non-exclusionary criteria used to evaluate the final suitability of each reintroduction site is shown in Table 3. Summarised values obtained from rating curves illustrating relationships between the quantity of the non-exclusionary criterion and a suitability score (0–1) are shown in Table 4. Final suitability values for eight different nominated reintroduction sites which are a product of weights and ratings of seven non-exclusionary criteria are shown in Table 6. This table shows the ranked suitability score for all sites based on numerical values obtained from the suitability assessment equation. The highest suitability value belongs to S5 with scores of 0.89 (Fig. 5, Table 6).

- Field studies

Finally, in selected sites with ranked suitability values (Fig. 5), the suitability of different potential reintroduction sites for *N. derjugini* was further assessed against several criteria related to water quality (PH, DO, turbidity, electrical conductivity, water discharge), special heterogeneity (food availability, length and width of water body, permanence of water body, presence of potential shelter sites and type, visible pollution, predator, shade) and habitat parameters (altitude, land use, temperature) along various streams (Table 7). Fifteen water bodies (springs, first order streams and karst seepage) were initially examined for the reintroduction site. Shelmav stream (N35°00’56.5”; E046°26’41.4”) in Khaneghah villages as a potential site for reintroduction of *N. derjugini* was selected based on a group of criteria to determine suitability for receiving founder stock (Fig. 5, Table 7). Most importantly, selection of this site was based on the degree of protection it afforded from predators, on its close vicinity to a macroinvertebrate community, its high altitude and cold water, isolation

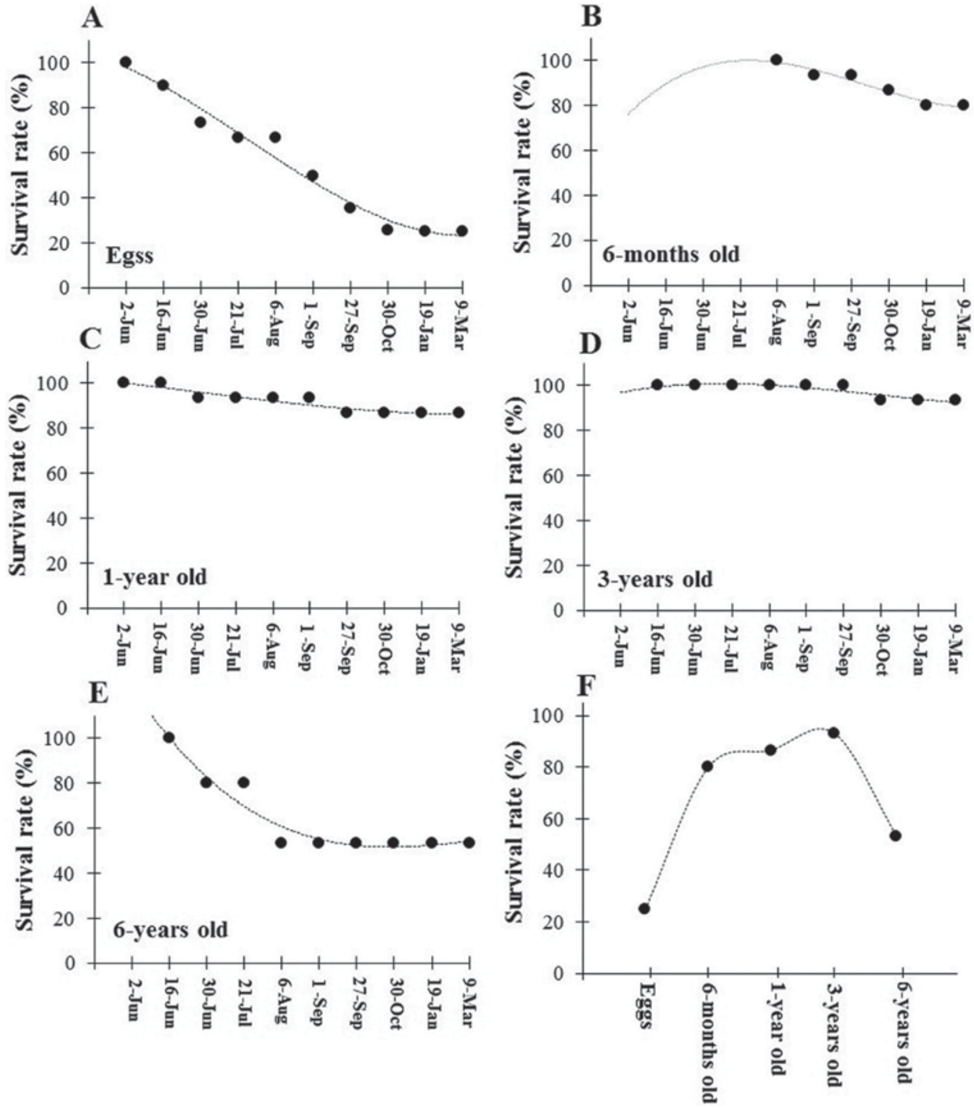

Figure 6. The survival rate (%) of different age class including **(A)** eggs, **(B)** larvae with six-months old, post-metamorphic with **(C)** one-year and **(D)** three-years old, an adult with **(E)** six-years old of *N. derjugini* reintroduced to the Shelmav stream during June 2017 until March 2018. The survival rate (%) at the end of the experiment **(F)**.

from human disturbance, lack of pollution and relative security from current and future habitat threats which might result from land-use alteration (Table 7).

Survival rates

Following selection of Shelmav Stream as a suitable site for reintroduction, eggs and various age classes of *N. derjugini* reared in the captive breeding facility were introduced in meshed bags in the reintroduction site. A number of individuals released and size of freshly laid eggs, and the total length of larvae with six-months old, post-metamorphic with one-year and three-years old, and adult with six-year old *N. derjugini* are shown in Table 8. The survival rates of different age class of *N. derjugini* reintroduced to the Shelmav Stream on 2 June 2017 to 9 March 2018 are shown in Fig. 6 A-E and Table 8. Over 10 visits to the site before and after overwintering, the newts with the three-years old had the highest survival rates (93.33 %), (Fig. 6D). In contrast, the eggs had the lowest survival rates (25 %), (Fig. 6A). The survival rate for six-months old larvae (Fig. 6B), the one-year old newts (Fig. 6C), and six-years old adults (Fig. 6E) were 80 %, 86 % and 53 %, respectively, at the end of the experiment (Fig. 6F, Table 8).

DISCUSSION

We assume that the ages of *N. derjugini* estimated indirectly from the number of the lines of arrested growth (LAGs) were accurate. This method has been used in an area with a cold temperate climate with over two months of freezing days in winter (Sharifi & Assadian, 2004). A similar method has been used by Uzum and Olgun (2009) and Kutrup et al. (2011) to estimate amphibian ages in Turkey close to the present study site. Several authors have supported the suggestion that one LAG equals one year by following known individual amphibians in the field (Buhlmann & Mitchell, 2000; Francillon-Vieillot et al., 1990; Gibbons & McCarthy, 2006) or under laboratory conditions (Kumbar & Katti, 2004). However, there are reports of inaccuracy in the skeletochronological estimates for oldest age class (13-15 years) when

Table 4. Grading values obtained from seven rating curves for seven no-exclusionary criteria used to evaluate relative suitability of selected areas by overlaying. Pattern is a general configuration of a rating curve describing relationship between each criteria and relevant suitability score ranging from 0 to 1.

No	Criteria	Pattern	Grading values
1	Conservation integrity	Linear	Weak=0.25-0.50, Average=0.50-0.75, Excellent=0.75-1
2	Habitat suitability score	Linear	0-20=0, 20-40=0.25; 40-60=50; 60-80=0.75; 80-100=1
3	Number of newt localities	Linear	0=0, 1-10=0.0.5; >10=1
4	Stream density	Linear	0-0.007 Km2=0, 0.007-0.01 Km2= 0.50, 0.01-0.32 Km2=1
5	Distance to protected area	Linear	>50 Km=0, 25-50 Km= 0.50, 0-25 Km=1
6	Villages density	Linear	3.68-10.94 Km2=0, 1.18 -3.68 Km2= 0.50, 0-1.18 Km2=1
7	Distance to main road	Linear	0-10 Km=0.5, 10-20 Km= 0.75, >20 Km=1

Table 5. A static life table for *N. derjugini* population in Kavat stream in western Iran. Number of live newt sampled at different age x (nx) and the proportion surviving at the start of age x (l_x) for both sexes of adult (over three years). Mortality rates (q_x) are obtained from the current reintroduction program.

Age class (x)	n_x	l_x	q_x
0-0.5	-	-	1-0.25
0.5-1	-	-	1-0.80
1-2	-	-	1-0.86
2-3	-	-	-
3-4	8	0.098	1-0.93
4-5	14	0.172	-
5-6	8	0.098	1-0.53
6-7	14	0.172	-
7-8	14	0.172	-
8-9	7	0.086	-
9-10	7	0.086	-
10-11	3	0.037	-
11-12	3	0.037	-
12-13	2	0.024	-
13-14	1	0.012	
	81	1	

compared to actual age (Eden et al., 2007). The reduced accuracy of skeletochronology for age determination may be the result of extensive resorption that occurred throughout the amphibian's life, resulting in a gross underestimate of the age of older individuals and in long-lived species (Sullivan & Fernandez, 1999). Double LAG can also occur as a result of a double cycle of annual activity such as hibernation and aestivation (Caetano, 1990). Despite these drawbacks, skeletochronology is a quick and widely used method for determination of age in temperate regions. Eden et al. (2007) have reported that over 100 authors have used this method to determine the age of various species of reptile or amphibian.

In this study, data obtained from skeletochronological analysis indicate that unlike its small body mass *N. derjugini* is a long-lived animal with a maximum age reported up to 13 years. Average and standard error of longevity for 81 male and female *N. derjugini* is 6.44±0.27 years. The highest longevity reached to 13 years for males and 12 years for females. Analogous life spans have been reported in other amphibian species, such as *Ambystoma tigrinum nebulosum*, 15 years (Eden et al., 2007), *Neurergus kaiseri*, 14 years (Farasat & Sharifi, 2016). Also, the minimum number of LAGs found in mature males and females shows that both male and female *N. derjugini* are potentially able to reproduce in their third breeding season. Consistent with our results, Farasat and Sharifi (2016) reported the age of sexual maturity for *N. kaiseri* to be four year. Üzüm (2009) has reported the age of sexual maturity for *Mertensiella caucasica* to be four year whereas in some populations of *Triturus karelinii* the age of maturation is three year (Olgun et al., 2005).

Assessing the suitability of a habitat prior to the release of animals is vital (Lawrence & Kaye, 2011; Van Liefferinge et al., 2018). This is particularly important for species or life stages that are unable to disperse long distances. For any species, the long-term consequences of habitat choice for survival and reproductive success define the ultimate quality of the habitat (William et al., 2007). Also, animals have adapted to consider habitat quality not only based on accessible resources but also by using secondary environmental or structural cues that have association with the final habitat quality, such as predation risk (Rantanen et al., 2010). Shelmav Stream as a potential site for reintroduction of *N. derjugini* was selected based on a group of exclusionary criteria to determine suitability within an area defined by a presence-only model (MaxEnt) (Sharifi et al., 2017). The present study demonstrates that combining MaxEnt, GIS and MCDA provides a practical and effective framework for prioritising and ranking landscapes for a reintroduction site for *N. derjugini*. In particular, the MCDA developed for this study was useful in addressing various issues for which there were no available spatial data as well as to incorporate human judgment into the selection process. We conclude that the present study provides a practical approach toward the combination of MaxEnt, GIS and MCDA in providing a series of ranked areas and allowed managers and other stakeholders in creating a reintroduction site. New spatial data or hypotheses can be easily integrated into current GIS-MCDA exercise. This study, also, was able to provide a model study in an area with inadequate spatial data that could be used for other species where there is a need to establish a reintroduction site.

Sarrazin and Legendre (2000) studied a demographic approach to reintroduction of a long-living bird, Griffon Vultures (*Gyps fulvus*), and documented that the release of adults in comparison with young is the most effective. Contrary to long-live species such as Griffon Vultures, short-lived species release costs can be lower. It has been suggested that captivity until the age at sexual maturity

Table 6. Suitability scores for eight nominated reintroduction site (S1-S8) evaluated based on eight exclusionary and seven non-exclusionary criteria.

Criterion	S1	S2	S3	S4	S5	S6	S7	S8
Conservation integrity	0.06	0.12	0.12	0.12	0.25	0.25	0.18	0.12
MaxEnt suitability score	0	0	0.05	0.15	0.20	0.15	0.20	0.05
Number of newt localities	0	0	0	0	0.08	0.08	0	0
Stream density	0.06	0.09	0.06	0.06	0.13	0.13	0.13	0.06
Distance to protected area	0.11	0	0	0.05	0.11	0.05	0.05	0.05
Villages density	0.04	0.09	0.04	0.04	0.09	0.04	0.04	0.09
Distance to main road	0.03	0.03	0.03	0.04	0.03	0.03	0.06	0.03
Suitability score	0.30	0.33	0.30	0.46	**0.89**	0.73	0.66	0. 40

Table 7. Several characteristics of aquatic environments in 15 first order streams nominated for reintroduction site including geographic position, altitude, food availability (adequate-inadequate), pH, salinity, DO, turbidity, dissolved oxygen water, temperature, discharge (Permanent/Ephemeral), and presence of natural predator (+).

Waterbodies	Position (l/s)	Altitude (m.s.l)	Food available	*N. derjugini* sighted (S)/ not sighted (N)	pH	Salinity (EC; µS/cm)	Turbidity (NTU)	Dissolved oxygen (mg/L)	Temperature (°C)	Permanent (P) /Ephemeral (E)	Predator		
											snake	crab	bufo
Kavat	N34°52'41.2" E046°30'34.60"	1560	Adequate	S	7.8	0.372	1	7.70	12	P	_	_	_
Ghorighale	N34°53'12.5" E046°29'18.7"	1480	Adequate	N	7.58	0.40	18	5.8	13	P	_	_	_
Zali	N34°59'01.0" E046°28'41.0"	1777	Inadequate	N	7.26	0.36	1	6.7	12.5	P	_	+	_
Karvansara (up)	N34°57'41.4" E046°26'45.9"	1819	Adequate	N	7.27	0.34	3	7.4	14	P	_	+	_
Karvansara (down)	N34°57'29.5" E046°26'33.6"	1759	Inadequate	N	7.4	0.32	3	5.2	13	E	_	_	+
Gholani	N34°54'14.0" E046°27'27.9"	1461	Adequate	S	7.8	0.42	3	6.6	13.5	P	_	_	+
Baiangan	N34°57'55.4" E046°18'01.4"	1334	Adequate	N	7.26	0.39	1	7.26	14	P	+	+	+
Tazeh abad	N34°57'45.8" E046°26'01.3"	1761	Adequate	N	7.30	0.39	1	7.46	15	E	_	+	_
Shikhahmad	N34°57'56.5" E046°27'01.4"	1882	Inadequate	N	6.88	0.30	1	7.22	14	E	_	_	_
Serajgah	N35°11'02.7" E046°14'43.8"	1469	Inadequate	S	7.13	0.37	2	6.09	15	P	_	+	_
Berno	N35°10'57.3" E046°14'47.0"	1464	Adequate	N	7.15	0.31	1	6.00	14	P	_	+	_
Kariz	N35°11'13.5" E046°14'37.7"	1558	Adequate	N	7.18	0.40	1	5.29	14	P	_	_	_
Shelmav	N35°00'56.5"; E046°26'41.4"	1713	Adequate	S	7.04	0.36	1	7.80	10	P	_	_	_
Nosod	N35°10'38.7" E046°12'06.2"	1355	Inadequate	N	7.62	0.32	1	5.70	16.5	P	_	+	_
Mivan	N34°57'29.5" E046°26'33.6"	1650	Adequate	N	7.20	0.36	1	7.69	12.50	P	_	_	_

Table 8. The number of individuals released and size of freshly laid eggs, and the total length of larvae with six-months old, post-metamorphic with one-year and three-years old, and adult with six-years old *N. derjugini* reintroduced into Shelmav stream.

Age class	No. released	Mean ± SD (mm)	Survival rate (%)	SE	LCI-UCI %95 CI
Egg diameter	30	8.84 ± 1.86	25	7.86	8.92-41.07
Larvae with 6-months old	15	78.63 ± 6.81	80	10.69	57.07-102.92
Post-metamorphs with 1-year old	15	92.87 ± 5.30	86.66	9.08	67.18-106.15
Post-metamorphs with 3-years old	15	139. 43 ± 4.20	93.33	6.66	79.03-107.63
Adult with 6-years old	15	150.28 ± 8.18	53.33	13.33	24.73-81.93

CI= Confidence Interval, LCI= Low CI; UCI= Upper CI can constitute a useful period in which the animal can acclimatise to the release area to achieve a comfortable release, and to reduce the stress causing short-term release costs (Bright & Morris, 1994). In the same way, this period can be used to complete the preparatory phase of habitat restoration, a crucial period for reintroduction success (Kleiman, 1989), and to educate local populations in order to increase conservation efficiency (Reading & Kellert, 2002). Also, another benefit of releasing mature individual is that the suitability of the selected habitat or the ability of released individuals to withstand and breed can be checked immediately after the release. This may not be the case for young individuals because of delayed maturity (Sarrazin & Legendre, 2000).

Generally, newly reintroduced individuals can suffer high mortality, as a result of the absence of natural selection during the captive phase or adaptation to life in captivity (Canessa et al., 2014). In several studyies reintroduced adults have shown abnormally high dispersal ability (Le Gouar, 2012). Based on photographic identification method average minimum distance covered by recaptured individuals indicates that the home range of *N. derjugini* during the breeding season when the newts live exclusively in the water was estimated to be 230 m^2 (Sharifi & Afroosheh, 2014). This small home range indicates that the chance of dispersal of reintroduced newts may not be very high. Moreover, data obtained in present study suggest that early support of eggs and larvae in mesh enclosure can progressively increase the survival rates in early stages of *N. derjugini* in early stages of life.

There is some information available on reproduction and captive breeding of *N. derjugini* that may be useful in optimising the release strategies for reintroductions of this species. Based on a study in the captive breeding facility for *N. derjugini* female newts may produce 100-150 individual eggs (Sharifi & Vaissi, 2014; Vaissi & Sharifi, 2018). The egg stage lasts 3–4 weeks; according to the conditions of temperature, food levels, density and water level, the larval period 4–16 months (Vaissi & Sharifi, 2016a, 2016b), reaching metamorphosis (loss of gills) with SVL approximately 46.13 to 78.09 mm. At this stage young metamorphs leave the water (Vaissi & Sharifi, 2018). During larval growth, SVL increases in a linear fashion, with respective growth rates of 1.73 mm/day (Vaissi et al., 2018). The level of cannibalistic behaviour changed as the larvae grew, from a low level during the first four weeks, peaking from weeks seven to 12, and then dropped during weeks 14–52 (Vaissi & Sharifi, 2016b). Also, overwinter mortality in *N. derjugini*, in a small spring enclosure was estimated from July to September 2012. This study demonstrates that post-metamorph captive-bred *N. derjugini* released into the wild can survive to the second growing season (Sharifi & Vaissi, 2014; Vaissi & Sharifi, 2018).

Neurergus derjugini has a slow growth rate with sexual maturation at age three. This indicates that if it was found appropriate to release individuals at the adult stage, newts would have to be sustained in captivity for a longer period. However, maturation at age three slows down the build-up of a captive population available for reintroduction and increases the expenditure per released newt. Moreover, if it was decided to release captive bred newts at higher age they live in the captive facility for a longer time, and adaptation to the captive life may cause negative impacts on the capability of the reintroduced individuals (Vaissi & Sharifi, 2018). The present experimental reintroduction in meshed enclosures demonstrated that six-months old larvae to three-year old captive-bred adult *N. derjugini* released into the wild can overwinter into the second growing season with high survival rate (≥80 %) and may be the best choice for a reintroduction plan.

CONCLUSION

In studying the reintroduction of the yellow spotted mountain newts in western Iran, we learned that integration of MaxEnt, GIS, MCDA and skeletochronology data with trial reintroduction results can provide scenarios for a typical reintroduction. In an area with a stable-age-structure of host population, or in similar habitat that have lost most of its population, reintroduction may involve releasing a captive population of similar age structure. This strategy requires the provision of adequate captive born individuals of different age classes, and is a long-term and expensive programme. A short-term and inexpensive programme involves a non-distributed captive bred population. The present study showed that with present surviving values some 650 fertilised eggs can produce 100 mature newts in mesh bags in three years. Reintroduction of 136 six-month old larvae, 116 one-year old juveniles, 107 two-year old, and 100 three-year old newts, the population can reach to 100 matured newts in 2.5, 2, 1 years, and directly at the same year, respectively.

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