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Communication

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The soft-release of captive-born Kaiser's Mountain Newt *Neurergus kaiseri* (Amphibia: Caudata) into a highland stream, western Iran

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PLATINUM OPEN ACCESS

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Abstract: Captive breeding and reintroduction programs are important conservation tools and are used for increasing the number of plant and animal species worldwide. The endemic Kaiser's Mountain Newt *Neurergus kaiseri* is listed as Vulnerable on the Red List by the International Union for Conservation of Nature (IUCN) and is amended to Appendix I of the Convention of International Trade on Endangered Species (CITES). In the present study, in order to learn about the survival ability of captive-born newts of *N. kaiseri*, we conducted a trial translocation of 15 two-year-old captive-born *N. kaiseri* into the highland stream in Sartakht Village, western Iran. The survival rate of these newts were determined in two stages, involving early acclimatization in mesh bags and direct release in a highland brook. In 12 surveys to the translocation site, a total of 86 individuals were identified during spring and summer. The average survival rate during the acclimatizing phase was 98 ± 0.04 %, while an average survival rate of 12 ± 0.04 % was obtained when the newts were released in the brook. Applying an average diurnal detection probability obtained for the Yellow Spotted Mountain Newt *Neurergus derjugini*, the overall survival rate in September when newts began the autumn withdrawal was 13%. These findings demonstrate that captive-born *N. kaiseri* released into the wild in controlled conditions can survive during spring and summer and provide information for future reintroduction plan of this species.

Keywords: Captive breeding, conservation, CITES, reintroduction, trial translocation, threatened species.

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Author contribution: TS was responsible for field and laboratory works and preparation of the manuscript. VA reviewed the manuscript. MSH conducted field surveys and reviewed and approved the manuscript.

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INTRODUCTION

The list of species categorized as Vulnerable, Endangered, or Critically Endangered has nearly doubled in the past two decades, and 20,000 species are estimated as endangered species on the verge of extinction (Estrada 2014). The release of captive-born individuals is being increasingly used as an important tool for the restoration of endangered and threatened species around the world (Seddon et al. 2007; Okuyama et al. 2010). The reintroduction of captive-bred animals for establishing a viable population often fails due to a range of reasons (Mathews et al. 2005), such as inability to avoid predators, a lack of searching for food and inability to process food, inability to find shelter, lack of predatory abilities, loss of social interactions with conspecifics and orientation in a complex environment (Okuyama et al. 2010).

Various studies conducted on reintroduced species have shown that different environmental and biological factors contribute to the success of reintroduction projects. One of these factors is the type of release; hard (direct) or soft (indirect) release (Serangeli et al. 2012). Based on the hypothesis that soft-release methods will improve the success of reintroduction, they are often included in reintroduction protocols (Moehrenschlager & Macdonald 2003). A common soft-release method is delayed release (or acclimatization) that prevents high rates of mortality that often occur immediately after release (Dickens et al. 2010). In this technique, individuals are placed in a protective enclosure at the release site, so that they can acclimatize to the new environment gradually before the release (Sutherland 2000). In addition, long-term food availability, habitat suitability and the season of release are other factors for the success of reintroduction (Jule et al. 2008; Serangeli et al. 2012).

Over 32% of amphibian species have been known as globally threatened species since the 1970s (Stuart et al. 2004). Different factors including pollution, over-consumption, habitat loss, climate change, and disease (both fungal and viral) play a role in these declines. Therefore, captive breeding programs are used increasingly to counter extinctions of wild populations of frogs and salamanders (Gascon et al. 2007; Bodinof et al. 2012). The drawbacks associated with captive breeding may be less important in amphibian species due to several life history attributes such as small body size with low space requirements, rapid growth and high fecundity (Kinne 2004; Sharifi & Vaissi 2014). There are several reasons to translocate animals that could generally be assigned to experimental research, to mitigate human-wildlife conflict and conservation or population re-establishment (Germano & Bishop 2009). The aim of the experimental reintroduction or research is to survey the best conditions for captive-bred survival in a release site and to improve reintroduction programs in the future (Liu et al. 2016). As a result, an experimental approach can help to provide appropriate guidelines for the success of the reintroduction program (Griffiths & Pavajeau 2008; Santos et al. 2009; Roe et al. 2010).

Kaiser's Mountain Newt *Neurergus kaiseri* is a species that is endemic to the southern Zagros Mountains with a restricted distribution in the Lorestan and Khuzestan provinces. Rapid declines due to its highly fragmented breeding habitat and also because it occupies a small range during its reproductive period implied the extinction of this newt in the wild and is pointed out to be one of the threatened species in Iran (Sharifi et al. 2009). Until recently, studies showed that localities from only four streams (in a single catchment area) increased to 40 new geographical localities. Therefore, this species was listed as Vulnerable by IUCN in 2017 (Mobaraki et al. 2014). Furthermore, this species has been listed in Appendix I of the Convention of International Trade on Endangered Species (CITES, https://www.cites.org/eng/app/appendices.php, Sharifi et al. 2009).

A conservation management plan funded by the Mohamed bin Zayed Species Conservation Fund was initiated for The Yellow Spotted Mountain Newt *Neurergus derjugini* (= *Neurergus microspilotus*) in 2010. Part of this plan included the development of a captive breeding facility (CBF) at Razi University, Kermanshah, Iran (Vaissi & Sharifi 2015). Several laboratory studies in the CBF on *N. derjugini* provided information on growth and development (Vaissi & Sharifi 2016a,b), cannibalism (Vaissi & Sharifi 2016b), ontogenetic changes in spot configuration (Vaissi & Sharifi 2017), a trial reintroduction of captive-bred newts (Sharifi & Vaissi 2014; Vaissi & Sharifi 2018), and comparing the predatory impact of captive-bred and free-living newts (Salehi & Sharifi 2019). Also, this project initiated a program for captive breeding and field studies of the Kaiser's Mountain Newt (Image 1A) aiming to provide critical information for a conservation management plan. These studies included sexual size dimorphism (Sharifi et al. 2012), histomorphological study (Parto et al. 2013; Parto et al. 2014b), reporting chytridiomycosis (Sharifi et al. 2014), and red-leg syndrome (Parto et al. 2014a), delimiting the species range (Sharifi et al. 2013), ageing and growth of species (Farasat & Sharifi 2016), reproductive morphology and sperm storage (Parto et al. 2015), and population genetic structure (Farasat et al. 2016).

Here we will use translocation as a term referring to the release of individuals from captive origins to areas without conspecifics into the wild for experimental evaluation of post-release survival of captive-reared Kaiser's Mountain Newts. Much of our discourse on translocation here will focus on the captive breeding management and therefore on captive-release programs. However, there was no monitoring work on the survival of the captive-born Kaiser's Mountain Newts into the wild so, here we describe a trial translocation of this species. For this purpose, the experimental release was carried out in two stages during spring and summer. The first stage involved the indirect release of individuals into the environment for acclimatization, and in the second stage, the newts were released directly into the environment.

The main aims of our trial translocation were: (i) whether captive-born newts could survive in the natural habitat during the acclimatization phase, (ii) to determine whether acclimatized newts would be observed at the release site after free release, and (iii) whether reintroduction of captive-born newts could be an effective conservation strategy for the recovery of a viable population in the future.

MATERIALS AND METHODS

Captive breeding

The previous ex situ conservation program on *N. derjugini* and their reintroduction provided relevant experience and information for the current work. In the spring of 2014, the first gravid females (SVL: 173.9–174.2 mm) from Bozorgab Stream (32.933^{0}N & 48.466^{0}E) in the southern Zagros Mountains were transported to CBF. The gravid females were detectable by their swollen bodies. They were introduced together in one aquarium (75 × 45 × 35 cm) with a water level of about 9cm and with small pebbles. Also, the aquaria were filled with mosses and some aquatic plants for egg attachment. Immediately after egg laying, we introduced the eggs (due to cannibalism) into separate rearing aquaria with aerated water. The egg stage lasted 2–3 weeks and then in the first phase of their life they were motionless and attached their mouth regions to plants, stones or other solid objects. In this life cycle, they consumed their internal nutrient reserves and then were fed with Artemia egg and shredded blood worms *Glycera dibranchiate*. The larval period lasted 8–9 months, reaching metamorphosis (loss of gills) and in this stage the young postmetamorphs (mean SVL was 30 ± 0.59 mm and mean body mass was 1.53 ± 0.05 g) left the water and they stayed more in the terrestrial component of their habitat. They were fed with a series of gradually larger food items including blood worms, earthworm *Lumbricus terrestris*, and live mealworms *Teneberio molitor* until they reached the scheduled release size.

Selecting newts for release

We released 15 two-year-old individuals (Image 1B, mean SVL was 35.71 ± 2.46 mm and mean body mass was 1.61 ± 0.48 g) in 2016 because individuals of smaller sizes are vulnerable to predators and environmental factors. In addition, larger individuals acclimatize themselves to the captive condition and likely lose their normal behavior and function. Prerelease protocols included testing for diseases, normal behaviors, and responsiveness to stimulus.

Selecting of the release site

The selected site in the present study was a highland brook in Sartakht Village (34.766^{0}N & 47.150^{0}E) in western Iran (Figure 1, Image 1C), a small highland (1,600m) stream with permanent water discharge. This was partly selected because it was close to a private property to which we had easy and regular access.

Acclimatization and free release

We performed a soft-release reintroduction involving a period of acclimatization in the highland stream to allow the newts to adjust to the environmental conditions and avoid the impact of native predators such as crabs (*Potamon bilobatum*). The newts were placed in mesh bags (46 × 30 × 36 cm) and hid under plants in release site on three occasions (5 individuals per occasion) in 2016: 29 April, 6 May, and 13 May (Image 1D). Periods of acclimatization were 9, 10 and 11 weeks for three occasions. We fed newts with mealworms in the first week of acclimatization to the environmental conditions. The remaining newts from acclimatization period were directly released into the highland brook on 15 July 2016 (Image 1E).

Visual monitoring and detection probability

The newts were monitored on seven occasions when they were in their mesh bags until we made sure of their relative survival during the acclimatization period. Following 70 days of acclimatization, the newts were released in the stream and monitored by visual counts on five occasions until 16 September 2016 when the newts began the autumn withdrawal from the stream. Visual counts conducted by two observers walking along the stream banks between 10.00h and 13.00h where

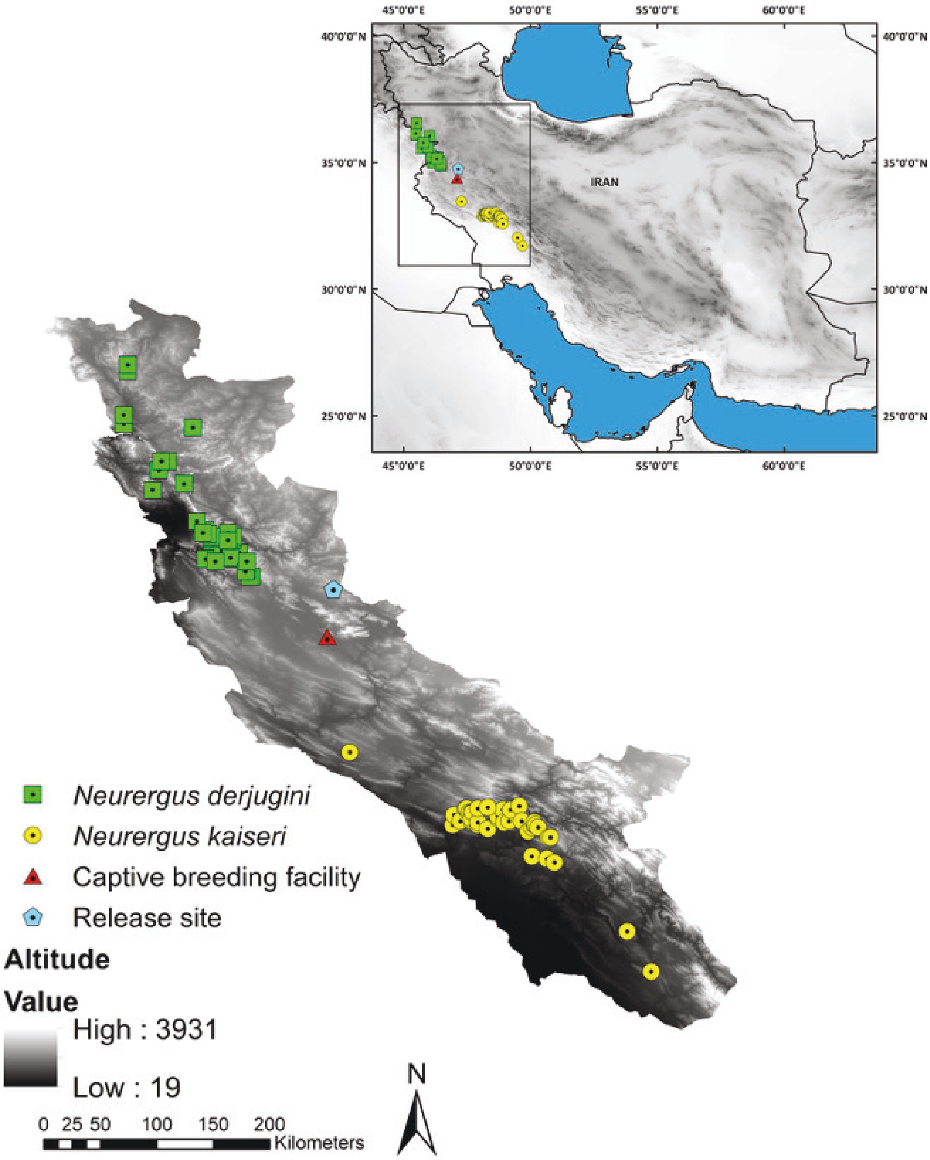

Figure 1. Distribution map of two species of mountain newts in western Iran, the Yellow Spotted Mountain Newt, *Neurergus derjugini* (green square) and the Kaiser's Mountain Newt, *Neurergus kaiseri* (yellow circle). The sites of the captive breeding facility (red triangle) and the translocating (blue pentagon) location between the ranges of the two species.

vegetation cover in the stream was dense and the search involved pursuing them under plants and rocks. Visual counts were conducted on 6 May, 13 May, 20 May, 3 June, 17 June, 1 July, 15 July, 22 July, 5 August, 19 August, 2 September and 16 September. For identification using coloration pattern recognition, we previously photographed the dorsal surfaces of the newts using a digital camera (Sony, DSCHX9V, 3.6V) on a tripod at a fixed height (30cm). After capturing, each newt was identified, measured, weighed, and the health status was checked.

Estimation of the survival rate of the released *N. kaiseri* involved the application of detection probability that was used for measuring *N. derjugini* (Sharifi & Vaissi 2014). This detection probability was obtained when a known number of newts were kept in several stone enclosures. The estimated average detection probability for *N. derjugini* was 0.61 ± 0.19 SD. We used the average detection probability as follows (Bailey et al. 2004): N = C/β where C, β, and N are the number of individuals counted, the probability of detection, and we adjusted the visual count respectively.

RESULTS

All of the individuals survived 100% during transportation from the CBF to the release site. Overall, we detected 86 individuals during 12 surveys. At the end of the acclimatization phase, we identified 13 individuals from 15 released newts within mesh bags making the survival rate (mean ± SD) in this period 98 ± 0.04 %. We observed two dead newts on 17 June. At the end of the acclimatization period, the body mass and SVL (mean ± SD) of the surviving newts were 1.69 ± 0.55 g and 38.33 ± 2.56 mm respectively.

In five visits from 22 July 2016 until 16 September 2016, only two released newts were found exactly at the point of release between the mosses and herbs around the stream on 19 August and 16 September 2016. The body mass and SVL of the newts on 19 August and 16 September were 1.56g, 33.36mm and 1.33g, 35.51mm, respectively. The survival rate (mean ± SD) in this period of 12 ± 0.04 % was considerably lower than the acclimatization phase. Based on the detection probability estimated for *N. derjugini*, the observed newts after final release were 13% of reintroduced newts. Table 1 describes the number, SVL, body mass, percent of surviving newts during acclimatization and free release periods and based on detection probability.

DISCUSSION

Release in controlled conditions has revealed the success of the acclimatized animals after release and the higher rates of individual survival (Mitchell et al. 2011). The soft-release of captive-born individuals has shown that this kind of release could provide an opportunity to adapt to the new environment, minimize mortality, and reduces the anxiety (Moseby et al. 2014). In the present study, we tested the survival rate in the acclimatization phase and during free release. The present trial translocation showed that that two-year-old captive-born newts would be able to survive in a natural habitat during acclimatization phase and the average survival rate of two year old newts in this period without humans was 98%. The high rate of survival during the acclimatization period is partly attributable to the protective mesh bags that avoid the impact of native predators and provides more time to adapt to natural conditions. Also, a similar translocation by Sharifi & Vaissi (2014) on *N. derjugini* that is a closely related species to *N. kaiseri* had demonstrated that young captive-born newts (in the relatively protected enclosure) can survive to the second growing season in the wild.

The survival rate in the free release phase was 12% of the released individuals. The present trial translocation showed that the survival of two-year-old captive born newts in the mesh bags remained high in the natural

Table 1. Date and number of the released individuals of *Neurergus kaiseri* into the wild, the age, SVL and body mass of released individuals, number of observed individuals during acclimatization phase, percent of individuals observed after and before free release phase, percent of surviving individuals based on next surveys and an estimate of the survival of newts outside mesh bags based on detection probability (db).

Date	No. released	No. observed	SVL (mm)	Body mass (mg)	% observed	% survived	% survived based on dp
29.iv.2016	5	-	34.31 ± 2.23	1.24 ± 0.16	-		
6.v.2016	5	5	35.62 ± 2.57	1.48 ± 0.22	100%		
13.v.2016	5	10	37.21 ± 2.08	1.65 ± 2.11	100%		
20.v.2016	0	15	35.71 ± 2.46	1.61 ± 0.48	100%		
3.vi.2016	0	15	35.91 ± 2.35	1.57 ± 3.21	100%		
17.vi.2016	0	13	36.04 ±2.49	1.68 ± 3.10	87%		
1.vii.2016	0	13	36.55 ± 2.55	1.69 ± 3.50	100%		
15.vii.2016	13*	13	38.33 ± 2.56	1.69 ± 3.55	100%		
22.vii.2016	0	0			0%	15%	0 %
5.viii.2016	0	0			0%	15%	0 %
19.viii.-2016	0	1			8%	15%	13 %
2.ix.2016	0	0			0%	8%	0 %
16.ix.2016	0	1			8%	8%	13 %

Note: The star mark is the date of free release into the wild outside the mesh bags.

Image 1. A—Kaiser's Mountain Newt *Neurergus kaiseri* | B—two-year- newt in captive breeding facility | C—the release site of *Neurergus kaiseri* into the highland brook | D—acclimatization period in mesh bag | E—direct release to environment. © M. Sharifi & T. Salehi.

habitat during the acclimatization phase. We did not observe any newts on 22 July and 5 August, while in the next surveys two newts were found. Therefore, it is possible that more newts survived and we were not able to find them. Failure to find these newts is likely due to the small size of the released newts and the complexity of the environment. McFadden et al. (2016) encountered major difficulties in finding the Northern Corroboree Frog *Pseudophryne pengilleyi* after reintroduction, due to their small size and cryptic behavior. The study by Randall et al. (2016) on the Northern Leopard Frog *Lithobates pipiens* revealed another major difficulty in the reintroduction program: the low number of individuals released and complexity of the habitat. Also, Bell et al. (2010) carried out the release with low numbers (<30) of the Maud Island Frog *Leiopelma pakeka* and demonstrated that the risk of predators had probably reduced the success of translocation.

We observed that the newts were moving to the wet areas around the water and hiding under vegetation cover at the time of the release. In the next surveys after the free release, some individuals were found close to the release site. Studies show soft-releases can increase site fidelity that is a common aspect of reintroductions with many amphibians (Wanless et al. 2002; Attum et al. 2011). The newts that were observed on 19 August and 16 September after direct release were precisely hidden in the vegetation cover in the initial release site. Moreover, we needed the transmitter for study dispersal and home range of newts after reintroduction while the results show that the use of the transmitter in released individuals can lead to vulnerability and an increase in the likelihood of their death (Miloski & Titus 2008). In addition, studies on salamanders have used few external transmitters because of the movement of salamanders and the complexity of their habitat (Dervo et al. 2010); and internal transmitters have been used in larger species such as the Spotted Salamander *Ambystoma maculatum* (McDonough & Paton 2007) and Chinese Giant Salamander *Andrias davidianus* (Zhang et al. 2018). In a study that was conducted to investigate the survival of 22 Chinese Giant Salamanders after reintroduction the internal transmitter was used. It, however, took too much time for the salamanders to recover from surgery (they needed almost four months to fully recover). Furthermore internal transmitters only last for about one year, and it is difficult to replace expired transmitters with new units, thus longer monitoring plans could not be applied (Zhang et al. 2018). The use of the internal transmitter in this study was impossible due to the low number and small size of the individuals that would increase mortalities at the beginning of release. Sharifi & Afroosheh (2014) have been able to effectively use photographic identification method as a non-invasive method in *N. derjugini*, the sister species of *N. kaiseri* to determine the home range of this newt during a breeding season. The result of this study showed very small home range and high site fidelity of *N. derjugini.*

CONCLUSION

The present trial on the soft-release of two-year-old captive born Kaiser's Mountain Newts in spring and summer involving an acclimatization period and a free release phase showed a high survival rate in the first stage, and a lower survival rate in the second stage. There were major difficulties including small size, cryptic behavior and complexity of habitat, and low number of released newts during direct release. Possible reasons for the failure of translocation can be predation or lack of site fidelity after free release. We hope the findings of this experimental research help future reintroduction programs. We suggest a larger number of newts in a predator-free release environment in future translocations. Also, we encourage comparison of trained and untrained newts in soft-release strategies in future reintroductions. Information on all studies will open the door for a successful reintroduction of *N. kaiseri*.

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