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Limb Malformation in the Kaiser's Mountain Newt (*Neurergus kaiseri*), Zagros Mountains, Iran

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***Abstract.*—Considering that amphibian malformations can be linked to environmental quality, knowledge of such abnormalities is essential for understanding ecology, biology, and the health of populations. Among 400 male and 711 female Kaiser's Mountain Newts (*Neurergus kaiseri*) from the Zagros Mountains, Iran, we identified 41 abnormalities, including nine cases of limb deformities and 32 malformations. These malformations encompassed brachydactyly, ectrodactyly, ectromely (subcategory: amely), oligodactyly, polydactyly, polyphalangy (subcategory: schizodactyly), polymely (subcategory: polypody), and syndactyly. We identified malformations in approximately 2% of the Kaiser's Mountain Newts in the Zagros Mountains. This represents the first documented case of malformations in this vulnerable amphibian. The cause of these malformations and deformities in *Neurergus kaiseri* in the Bozorg-Ab habitat in the northern part of Khuzestan Province remains unknown. We recommend further research, including a focused study of parasite infection in this species within the Bozorg-Ab habitat, long-term habitat monitoring, and assessment of acaricides and other water pollutants at this site. Furthermore, we propose investigating malformations and conducting monitoring studies at other habitats of *N. kaiseri*.**

Key Words.—abnormality; anomaly; Caudata; deformity; limb regeneration; malformed amphibians; polydactyly; polymely; polyphalangy; Salamandridae

Introduction

Amphibians are highly sensitive to environmental changes and exhibit rapid responses to such changes; consequently, they serve as suitable bioindicators of ecosystem health (Simon et al. 2011; Balicka et al. 2020). Malformations in amphibians are often caused by genetic and developmental disorders and could be a sign of poor environmental quality (Balicka et al. 2020). Information about abnormalities can be an important component for understanding population ecology, biology, and health of amphibians (Romano et al. 2017). Tailed amphibians (Caudata; salamanders and newts), possess the ability to regenerate limbs, tails, and jaws (Mescher and Neff 2005; Lunghi et al. 2016). Regeneration processes of salamanders represent a very complex phase, during which several factors may trigger malformations (Lunghi et al. 2016). Amphibian malformations generally result from a biotic or abiotic disruption of normal regeneration (Williams et al. 2008). There are four major explanations of limb malformation in wild populations: pesticides, UV-B radiation, predator injuries, and parasites (Butler and Blum 1963; Johnson et al. 1999, 2006; Blaustein and Johnson 2003; Lunde and Johnson 2012). Using genetic data, Williams et al. (2008) concluded that neither inbreeding nor lack of genetic diversity are contributing factors to malformations. Mačát et al. (2015) indicated that malformations could be caused by hybridization, and high proportions of some newt malformations are found in hybrid zones (Arntzen and Wallis 1991).

Mountain newts (*Neurergus*) are the most diverse genus of the family Salamandridae in Mesopotamia. The Kaiser's Mountain Newt (*Neurergus kaiseri*) is endemic to the Central Zagros Mountains where mountainous streams and springs in Persian Oak (*Quercus brantii*) forests serve as its primary habitat located on the northern edge of the Great Karoon and Karkheh basins (Safaei-Mahroo and Ghaffari 2021). According to the Iranian Department of Environment, *Neurgus kaiseri* is an Endangered species, and according to the International Union for Conservation of Nature (IUCN) Red List, it is a Vulnerable species (IUCN 2016). Since 2010, this species has been listed on Appendix I of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) due to illegal trade (CITES 2010). Besides illegal trade, this iconic newt faces threats from water resource overexploitation and habitat destruction. Furthermore, acaricides and detergents used widely among nomads in the study area threaten its survival (Safaei-Mahroo 2014). Although cases of limb abnormalities have been reported in Eurasian newts (Jarvis 2011; Gatti and Sannolo 2014; Mačát et al. 2015), no published literature reports limb malformations of species in *Neurergus*. We report limb malformations in an isolated population of the Kaiser's Mountain Newt from the Zagros Mountains, Iran, including brachydactyly, ectrodactyly, ectromely (subcategory: amely), oligodactyly, polydactyly, polyphalangy (subcategory: schizodactyly), polymely (subcategory: polypody), and syndactyly.

Materials And Methods

Study site.—We conducted surveys in the Bozorg-Ab habitat within the Alvar-e-Garmsiri region in Andimeshk County, in the northern part of Khuzestan Province, Iran. This area is in a natural oak forest in the southwest part of the Zagros Mountains, at 32°56'41"N, 48°28'26"E, with an elevation ranging from 1,250 to 1,335 m. This region is surrounded by two large mountain walls in the southwestern Zagros. Bozorg-Ab habitat contains springs and streams with a length of approximately 0.5 km, which are used seasonally by Bakhtiari nomads as permanent sources of water. This stream supports one of the largest known populations of *N. kaiseri*.

Fieldwork and sampling methods.—We captured 400 male and 711 female *N. kaiseri* by hand or with dip nets in water bodies and along streamside habitats during a demographic study of this species in the breeding season (April to May) of 2019 (Safaei-Mahroo et al. 2023). As part of this study, we found abnormal adult individuals, including those with malformations and deformities. We used the terminology for malformation and deformity as defined by Johnson et al. (2001) and Henle et al. (2017b). We identified each malformation and deformity based on Meteyer (2000), Butler and Blum (1963), Johnson et al. (2001) and Henle et al. (2017b). We photographed the dorsal and ventral parts of most limb abnormalities using a Canon 60D camera (Canon Incorporated., Tokyo, Japan) with a 100mm L Macro IS lens (Canon, Incorporated) and a Nikon W300 camera (Nikon Group Companies, Bekasi, Indonesia) in its natural habitat. After photography, we released all adult newts back into their natural habitat.

Results

We examined 1,111 *N. kaiser* (400 males and 711 females) and detected 41 abnormalities in 33 individuals (2.97% of all individuals; Table 1). In 24 newts (2.16% of all captures) with limb abnormalities, we identified eight types of malformation: brachydactyly, ectrodactyly, ectromely, oligodactyly, polydactyly, polymely, polyphalangy, and syndactyly (Fig. 1). Additionally, we identified nine individuals with limb deformities (Table 1).

In comparison to males, females had a higher proportion of abnormalities. Of 711 females, 22 individuals (3.09% of all females) displayed 26 malformations, two deformities, and two stunted tails. Only one female had more than one malformed limb. We identified seven females with multiple malformations per limb. We found the following malformations only in females: brachydactyly (0.28%), polymely (0.28%), syndactyly (0.36%), and polydactyly (0.98%). Of 400 males, 11 individuals (2.75% of all males) had six malformations, two deformities, and three stunted tails (Fig. 2). No male exhibited more than one malformation.

Polyphalangy was the dominant malformation, followed by polydactyly, oligodactyly, syndactyly, ectrodactyly, brachydactyly, ectromely, and polymely (Table 1). We observed polyphalangy in eight newts (0.72%), occurring in both sexes, and at various parts of fingers and toes. These abnormalities included bifurcation of the distal phalange (Fig. 3); duplication of the proximal phalange (Figs. 3 and 4); duplication of the distal phalange (Fig. 5); triplication of the distal phalange (Fig. 5); bifurcation of proximal phalange in both the forelimbs and hindlimbs (Fig.

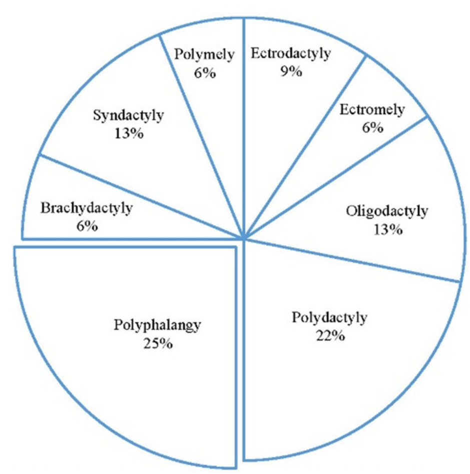

Figure 1. Percentage of types of malformations observed in the Kaiser's Mountain Newt (*Neurergus kaiseri*) in the Bozorg-Ab habitat of Iran.

Table 1. List of the number of abnormality observations with percentage of abnormalities in parentheses including malformation and deformity in the Kaiser's Mountain Newt (Neurergus kaiseri) in the Bozorg-Ab habitat of Iran. Terms adapted from Meteyer (2000), Johnson et al. (2001), and Henle et al. (2017b). The abbreviation FA = frequency of abnormality. One asterisk (*) indicates that we observed seven females (three forelimbs and four hindlimbs) with more than one malformation and two asterisks (**) indicate that we observed one gravid female with more than one malformation.

	Females		Males		
Abnormality Category: Description	Forelimb	Hindlimb	Forelimb	Hindlimb	FA (n, %)
Brachydactyly: Dwarfed one or more digits.	2 (0.28%)	0	0	0	2 (0.18%)
Ectrodactyly: Complete absence of one or more digits.	1 (0.14%)	0	0	2 (0.50%)	3 (0.27%)
Ectromely: Partial or complete absence of one or more limbs (subcategory: Amely).	0	1 (0.14%)	1 (0.25%)	0	2 (0.18%)
Oligodactyly: Fewer than normal number of digits but at least one digit present.	0	2 (0.28%)	1 (0.25%)	1 (0.25%)	4 (0.36%)
Polydactyly: Growth of one or more extra digits or parts of digits.	4 (0.56%)	3 (0.42%)	0	0	7 (0.63%)
Polymely: Growth of one or more extra limbs or parts of limbs (subcategory: Polypody).	2 (0.28%)	0	0	0	2 (0.18%)
Polyphalangy: Duplication of the phalangeal sets beside a normal finger or toe (subcategory: Schizodactyly).	5 (0.70%)	2 (0.28%)	1 (0.25%)	0	8 (0.72%)
Syndactyly: Partial or complete fusion of two or more digits.	0	4 (0.28%)	0	0	4 (0.36%)
Frequency of malformation	14	12	3	3	32
Total cases of limb malformation	14-3* = 11	12-4* = 8	3	3	32-7* = 25
Total individuals with limb malformation	19-1** = 18 (2.53%)		6 (1.50%)		24 (2.16%)
Limb deformities: Digit defects.	2 (0.28%)	0	1 (0.25%)	1 (0.25%)	4 (0.36%)
Tail stunted: Shortened tail.	2 (0.28%)		3 (0.75%)		5 (0.45%)
Total individuals with malformation and deformity	22 (3.09%)		11 (2.75%)		33 (2.97%)
All limb normal individuals	689 (96.91%)		389 (97.25%)		1078 (97.03%)
Total sampled	711		400		1111

5). We classified specimens with extra duplicated phalanges and digits that split or bend away from the normal pattern as schizodactyly, a subcategory of polyphalangy, following the definition provided by Henle et al. (2017b; Fig. 5).

Polydactyly, which we observed in seven cases (0.63%), occurred in various forms, including an extra finger over the metatarsal with duplication of the distal phalange (Fig. 3). We also found duplication of toes with partial fusion at the base (Fig. 4) and a second set of digits originating from the external part of the hindlimb, positioned laterally relative to the first four digits and consisting of only three digits (Fig. 4). In addition, we found a split foot with two sets of claws, each growing from the metatarsal and having an atypical number of digits and two extra fingers originating from the metatarsal on the palm of the forelimb (Fig. 4).

We observed four cases of oligodactyly (Fig. 3) and four cases of syndactyly (Fig. 4). The presence of syndactyly was always associated with other malformations. We found three newts with ectrodactyly (0.27%; Fig. 3). The rarest malformations were brachydactyly, ectromely (Fig. 3), and polymely (Fig. 5) with each occurring twice. Both cases of polypody, a form of polymely characterized by incomplete limb duplication, were observed in the left forelimb of females. We observed no fully formed duplicated limbs with fused proximal segments in our specimens. Consequently, we did not detect schizomely in the study area. During field surveys, we found Bedriaga's Marsh Frog (*Pelophylax* cf. *bedriagae*), Lorestan Melodious Toad (*Calliopersa luristanica*), and Savigny's Tree Frog (*Hyla savignyi*) sympatric with *N. kaiseri*, but we did not find any malformed individuals among those we encountered.

Discussion

We identified malformations in approximately 2% of the studied Kaiser's Mountain Newts in the Zagros

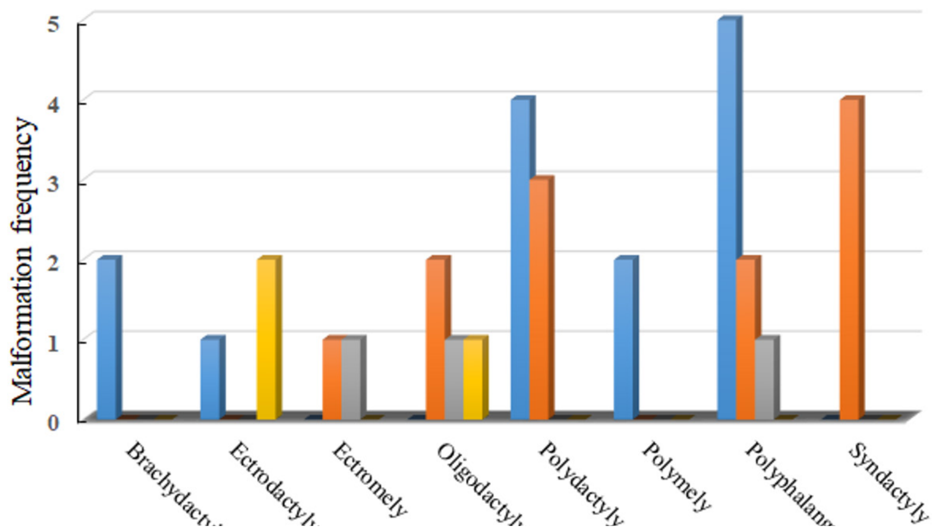

Figure 2. Frequency of forelimb and hindlimb malformation in male and female Kaiser's Mountain Newt (*Neurergus kaiseri*) individuals in the Bozorg-Ab habitat of Iran. Colors represent female forelimb (blue), female hindlimb (orange), male forelimb (gray), male hindlimb (yellow).

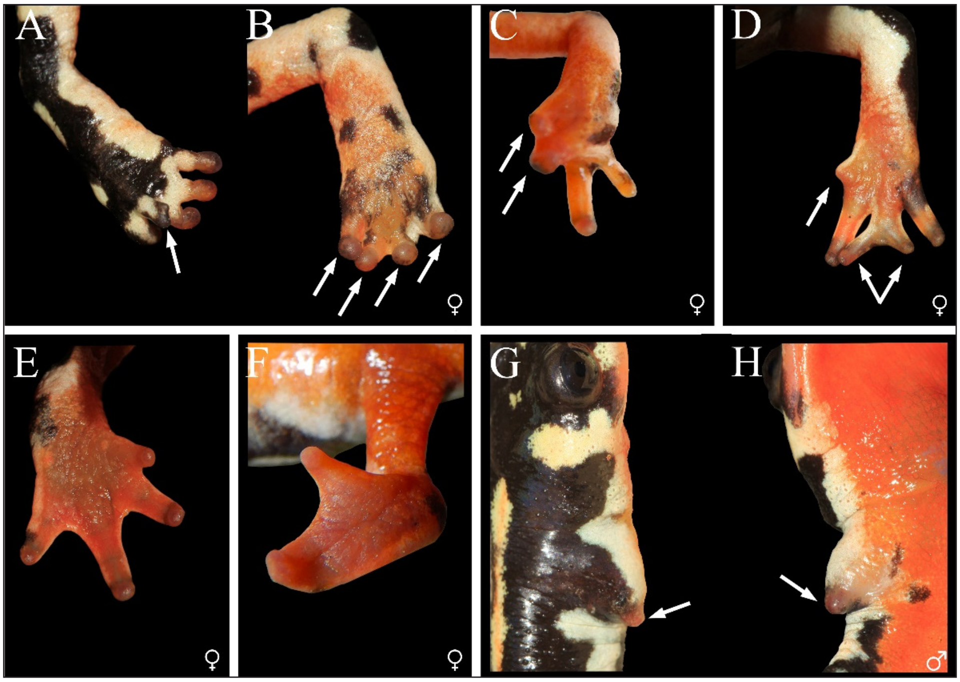

Figure 3. Malformation records in adult Kaiser's Mountain Newt (*Neurergus kaiseri*) in the Bozorg-Ab habitat of Iran; (A-B) Brachydactyly in all fingers with additional polydactyly and a polyphalangic fingertip at the extra digit (dorsal and ventral views of the right forelimb of a female). (C) Brachydactyly in the first and second digits (dorsal view of the right forelimb of a female). (D) Ectrodactyly (hypophalangy) in the 1st finger and polyphalangy in the third finger (ventral view of the forelimb of a female). (E) Oligodactyly with four toes (ventral view of the left hindlimb of a female). (F) Oligodactyly (ventral view of the left hindlimb of a female). (G-H) Ectromely with a small amount of limb tissue in the mid-bud blastemal stage (lateral and ventral views of the right forelimb of a male). (Photographed by Barbod Safaei-Mahroo).

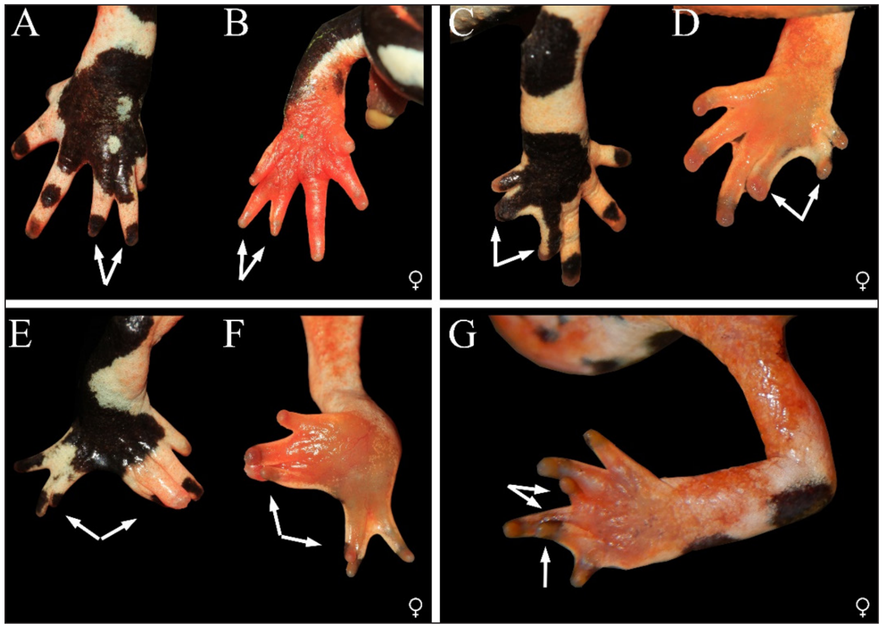

Figure 4. Polydactyly records in adult Kaiser's Mountain Newt (*Neurergus kaiseri*) in the Bozorg-Ab habitat of Iran: (A-B) Synpolydactyly with fused halves of the digits at the base of the fourth and fifth toes (dorsal and ventral views of the left hindlimb of a female). (C-D) Synpolydactyly between the 3rd and 4th toes and between the fifth and sixth toes (dorsal and ventral views of the left hindlimb of a female). (E-F) Hyperdactyly and digit swollen in secondtoe of the normal claw (dorsal and ventral views of the right hindlimb of a female). (G) Polydactyly with two extra digits on the palm of the forelimb and polyphalangy on the third digit (ventral view of right forelimb of a female). (Photographed by Barbod Safaei-Mahroo).

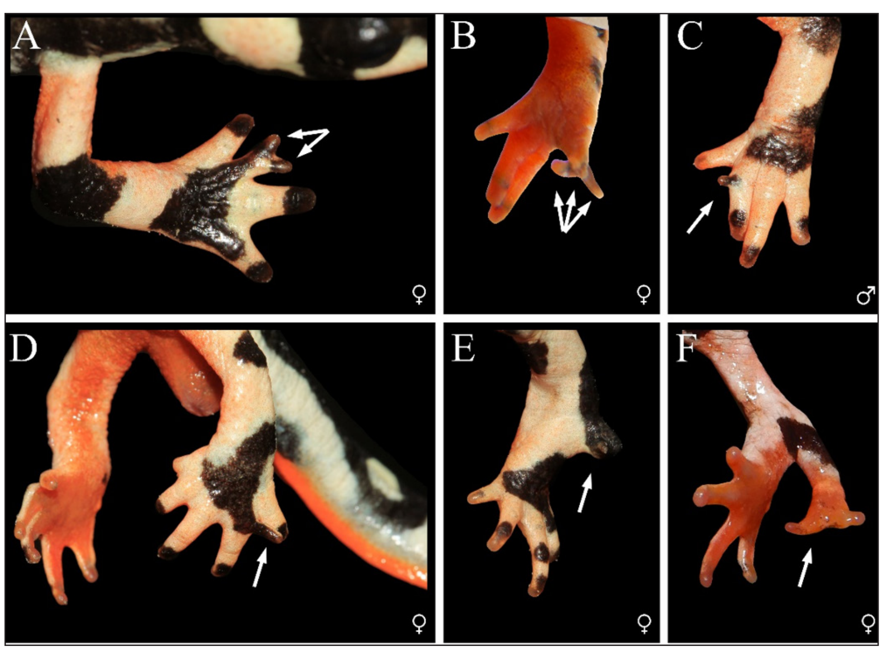

Figure 5. Polyphalangy, and polymely records in adult Kaiser's Mountain Newt (*Neurergus kaiseri*) in the Bozorg-Ab habitat of Iran: (A) Schizodactyly of the second digit at the fingertip (dorsal view of the right forelimb of a female). (B) Schizodactyly of the first finger at the fingertip with three phalanges (ventral view of the right forelimb of a female). (C) Schizodactyly, bifurcation on the side of the second digit (dorsal view of the left forelimb of a male). (D) Schizodactyly, bifurcation at the base of the fourth toe (dorsal view of the left hindlimb of a female). (E) A form of polymely (polypody) (dorsal view of the left forelimb of a female). (F) A form of polymely (polypody) (ventral view of the left forelimb of a female). (Photographed by Barbod Safaei-Mahroo).

Mountains. This represents the first documented case of malformations in this vulnerable amphibian. In our study, there was a dominance of malformed forelimbs over the malformed hindlimbs, with rates of 2.53% and 1.50% among all identified individuals, respectively. The malformations we observed in Kaiser's Mountain Newts included brachydactyly, ectrodactyly, ectromely, oligodactyly, polydactyly, polymely, polyphalangy, and syndactyly.

Previous studies have found limb malformations in other populations of newts, such as the Bosca's Newt (*Lissotriton boscai*), Sharp-ribbed Newt (*Pleurodeles waltl*), Palmate Newt (*Lissotriton helveticus*), and the Sharp-ribbed Newt (*P. waltl*), with incidences ranging from 2.38% to 4.30% (Zamora-Camacho 2020; Diego-Rasilla 2009; Laurentino et al. 2016). In the studied *N. kaiseri* population, 0.63% of newts were polydactylous. Polydactylous cases in different newt species have been identified in several populations of newts, including the Italian Crested Newt (*Triturus carnifex*), Japanese Fire-bellied Newt (*Cynops pyrrhogaster*), Marbled Newt (*Triturus marmoratus*), and the Smooth Newt (*Lissotriton vulgaris*), with reported frequencies ranging from 0.59% to 5.32% (Vershinin and Berzin 2018; Laurentino et al. 2016; Meyer-Rochow and Asashima 1988; Gatti and Sannolo 2014). We identified oligodactyly at a rate of 0.36% in *N. kaiseri*. In other caudates, including the Common Mudpuppy (*Necturus maculosus*) and *C. pyrrhogaster*, oligodactyly ranged from 0.004% to 1.22% (Meyer-Rochow and Asashima 1988; Hutt 1945).

The prevalence of ectromely, ectrodactyly, and syndactyly in *N. kaiseri* was 0.18%, 0.27%, and 0.36%, respectively. Among all types of malformations examined, only males with ectromely had higher prevalence than females. In *L. vulgaris*, Vershinin and Berzin (2018) reported rates of 1.22% for ectromely, 10.06% for ectrodactyly, and 0.6% for syndactyly.

In Kaiser's Mountain Newts, we identified 0.18% polymely. Meyer-Rochow and Asashima (1988) reorted 0.05% polymely in *C. pyrrhogaster*. These differences in malformation occurrence highlight the variability and frequency of limb malformations among different newt species. Understanding the prevalence of these limb malformations can help the identification of potential environmental factors contributing to such anomalies, which can help develop targeted and effective conservation strategies.

Possible implication for conservation and future research.—In amphibians, morphological deformities have a wide variety of causes, whether natural or anthropogenic, and possible interactions between stressors. Although no specific research studies have directly examined whether malformations threaten amphibian populations, the absence of evidence does not necessarily imply that there is no connection. It could be important to understand whether malformations contribute to ongoing declines (Lunde and Johnson 2012). Having a deformity may lead to impaired mobility, reduced food intake, increased susceptibility to predators and parasites, and ultimately result in high levels of mortality (Blaustein and Johnson 2003; Laurentino et al. 2016).

We do not know the cause of the malformations and deformities we found in 41 of *N. kaiseri* in the Bozorg-Ab habitat in the northern part of Khuzestan Province. There are several possible explanations for limb malformations in wild populations, all of which have the potential to contribute to their occurrence. The most common causes include ultraviolet-B radiation, predation attempts, parasite infection, and chemical contaminants (Johnson et al. 2024). Attempting to determine which factors caused amphibian malformations by focusing on only one of the possible causes will not yield a clear answer (Vershinin 1989). We did not observe injured Kaiser's Mountain Newts in Bozorg-Ab area. Solar UV-B (280–315 nm) radiation, possibly related to stratospheric ozone depletion, climate change, and deforestation (Blaustein et al. 2001; Londero et al. 2019), can cause malformations. The effect of UV on increasing the probability of abnormality has been well demonstrated (Butler and Blum 1963). According to Brown et al. (1994), UV-B levels near the forest floor are generally lower than those incident on the outer canopy. Furthermore, UV-B transmittance is highly dependent on canopy structure. Because our study area in the Bozorg-Ab habitat is a natural oak forest and *N. kaiseri* inhabits springs sheltered by the dense canopy of oaks, it seems likely that UV-B radiation does not have a significant impact on *N. kaiseri* malformations and deformities in this area.

Global warming, by raising water and air temperatures in microhabitats, enhances parasite activity and infectivity, leading to increased disease transmission among amphibians (Blaustein et al. 2012). Parasite infections also cause amphibian deformities (Kiesecker 2002; Johnson et al. 2006). Due to the widespread illegal trade of *N. kaiseri* in recent decades, newts collected from illegal markets, where they are kept in aquariums, have been returned to the natural habitat several times by the Iranian Department of Environment (DOE) in Alvar-e-Garmsiri habitats, prior to this study (DOE 2018), raising the possibility of parasite transmission. To date, there are no reports of parasitic infections of *N. kaiseri* in the study area.

Agrichemicals, such as endosulfan and carbaryl, can cause limb deformities and malformations in the larval stages of salamanders (Rohr et al. 2003). Our study area was surrounded by a mountainous oak forest. Because there are no villages or agricultural activities upstream, it is unlikely that rural or agricultural wastewater would enter this aquatic habitat. We did not conduct water quality analyses; however, in some parts downstream, nomads apply the acaricide to disinfect their livestock. Nomads in this area can obtain free cypermethrin EC 10% from the Nomadic Affairs Department by request the Veterinary Organization. Because it is free, nomads use it frequently. Cypermethrin pollution increases the incidence of malformations in amphibians (Agostini et al. 2010).

Although no parasitic infections have been studied in the study area, parasite infection and chemical contaminants may contribute to deformities observed in *N. kaiseri* in the Bozorg-Ab habitat. Additional research on the incidence of parasitic infection within this population is required to verify this claim. It is broadly thought that the prevalence of anomalies has increased in recent decades and there is an urgent need for well-designed long-term monitoring studies at sites where anomalies have previously been assessed (Henle et al. 2017a). There is a need to identify the main cause and origin of malformations in *N. kaiseri* in this area. We recommend further research, including a focused study of parasite infection in this species within the Bozorg-Ab habitat, long-term habitat monitoring, and assessment of acaricides and other water pollutants at this site. Furthermore, we propose investigating malformations and conducting monitoring studies at other habitats of *N. kaiseri*.

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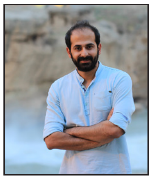

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