Comparing the predatory impact of captive–bred and free–living yellow spotted mountain newt (*Neurergus microspilotus*) on the larval green toad (*Bufotes variabilis*)

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Abstract

*Comparing the predatory impact of captive–bred and free–living yellow spotted mountain newt (*Neurergus microspilotus*) on the larval green toad (*Bufotes variabilis*).* Captive breeding of endangered species is an important conservation tool, but it is not clear how long–term captive breeding can influence fitness attributes such as predatory ability. We experimentally investigated the predatory impact of adult captive–bred newts (CBN) and adult free–living newts (FLN) on the survival and growth of larval green toad (*B. variabilis*) in four predator density treatments containing none, one, two, or three newts. FLNs performed a rapid density–dependent predation, yielding average survival rates of tadpoles in no, low, medium, and high densities to 81 %, 74 %, 60 % and 17 %, respectively. CBNs had an average lower predation rate on *B. variabilis* tadpoles with a decrease in survival rate of tadpoles to 83 %, 81 %, 82 % and 77 % for 0, 1, 2 and 3 predator treatments, respectively. However, contrary to FLNs, they exhibited a significant increase in predation rate with time from 0.37 to 0.60 tadpoles per day. In addition, the growth rate of tadpoles reared with predators for the FLN group was significantly higher than the growth rate of tadpoles reared in control containers without the predator. In conclusion, our findings suggest that exposing captive–born adult yellow spotted mountain newts to their potential prey enriches the environment, and may be a useful approach in the development of more efficient captive breeding and reintroduction programs for this highly endangered amphibian.

Key words: Captive breeding, Reintroduction, Predation, Endangered species, Tadpole, Conservation

Resumen

*Una comparación de la capacidad de depredación de los individuos nacidos en cautividad y en libertad del tritón del Kurdistán (*Neurergus micropillotus*) sobre larvas de sapo verde (*Bufotes variabilis*).* La cría en cautividad de especies amenazadas es un práctica importante en la conservación, pero no está claro si los programas de cría en cautividad a largo plazo pueden afectar a determinados atributos fundamentales de la eficacia biológica de las especies como la capacidad de depredación. En el presente estudio se estudian experimentalmente los efectos de la capacidad de depredación de tritones del Kurdistán adultos nacidos en cautividad (CBN) y de individuos obtenidos directamente de la naturaleza (FLN) en la supervivencia y el crecimiento de renacuajos de sapo verde mantenidos en cuatro tratamientos de densidad de depredadores, que contenían cero, uno, dos y tres tritones. El grupo de tritones FLN mostró una tasa de depredación rápida dependiente de la densidad de depredadores que conllevó que las tasas de supervivencia de los renacuajos en las densidades de control, baja, media y alta fueran, respectivamente, del 81 %, el 74 %, el 60 % y el 17 %. El grupo CBN tuvo una menor tasa media de depredación sobre los renacuajos de *B. variabilis*, cuya tasa de supervivencia disminuyó hasta el 83 %, el 81 %, el 82 % y el 77 % para los tratamientos con cero, uno, dos y tres tritones, respectivamente. Sin embargo, a diferencia del grupo FLN, esta tasa de depredación aumentó significativamente con el tiempo y pasó de 0,37 a 0,60 renacuajos por día. Por otra parte, la tasa de crecimiento de los renacuajos criados con depredadores del grupo FLN fue significativamente superior a la de los renacuajos criados en contenedores de control sin depredadores. Como conclusión, nuestros resultados sugieren que la exposición de individuos nacidos en cautividad del tritón del Kurdistán a sus presas potenciales enriquece el medio ambiente y puede ser útil para elaborar programas más eficientes de cría en cautividad y reintroducción de esta especie de anfibio muy amenazada.

Palabras clave: Cría en cautividad, Reintroducción, Depredación, Especies amenazadas, Renacuajo, Conservación

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Introduction

Captive breeding programmes have been used for a number of endangered species to save them from extinction, but the long–term outcome of these programmes has not always been satisfactory for a variety of reasons (Hedrick and Fredrickson, 2008). Some captive breeding programmes in amphibians have been characterized by poor nutritional requirements for growth (Pough, 2007), reduced natural behaviours such as foraging (including finding, identifying, acquiring, and handling food) (Burghardt, 2013), inactivity in the natural environment (Keulen and Janssens, 2017), inability to recognize natural foods (Olfert et al., 1993), a variety of diseases due to nutritional deficiency (Densmore and Green, 2007), chytridiomycosis infections (Parto et al., 2013), unsuccessful reproduction (Browne and Zippel, 2007), loss of social interactions (Rabin, 2003), variations in morphology of natural skin coloration (Ogilvy et al., 2012), loss of anti–predator response to predators (Kraaijeveld-Smit et al., 2006), a reduced immune response (Keulen and Janssens, 2017) and losing genetic diversity due to inbreeding (Zippel et al., 2011). The potential survival of captive–born amphibians after their reintroduction into the wild is one of the concerns (Michaels et al., 2014).

Although the above–mentioned studies cast doubts on the value of captive breeding and subsequent reintroduction as a conservation tool for threatened amphibian species (Griffiths and Pavajeau, 2008), there are always situations in which the captive breeding is the only conservation option available (Stuart et al., 2004). Harding et al. (2016) reviewed captive breeding programmes involving 213 amphibian species and found captive breeding affected various characteristics that could be taken into account in efforts to improve captivity management. Additionally, efforts to expand current understanding of ecology and behaviour of re–introduced species are growing (Pough, 2007). Moreover, research focusing on issues related to the release of captive–bred animals to the wild, such as acclimation to the new environments, pre–release health condition, genetic management, and long–term post–release monitoring, is greatly needed (Armstrong and Seddon, 2008). 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 and Seddon, 2008), and, especially in view of the practical irreversibility of many current threats to amphibians in their natural environments, which makes captive breeding and reintroduction indispensable conservation methods (Griffiths and Pavajeau, 2008; Harding et al., 2016).

The yellow spotted mountain newt (*Neurergus microspilotus*) is included on the IUCN Red List as a critically endangered species for many reasons: its very small area of occupancy in its breeding streams (< 10 km), fragmented habitats, continuing decline in the quality and extent of aquatic habitats, habitat degradation, drought, and the pet trade (Sharifi et al., 2009). A conservation management plan funded by the Mohamed bin Zayed Species Conservation Fund was initiated for this species in 2010. Part of this plan included the development of a captive breeding facility at Razi University, Kermanshah, Iran (Vaissi and Sharifi, 2015). The project also launched several field and laboratory studies aiming to provide critical information for the management plan. These studies include delimiting the species range (Afroosheh et al., 2016), feeding habits (Farasat and Sharifi, 2014), activity pattern and home range (Sharifi and Afroosheh, 2014), reports on emergent diseases such as chytridiomycosis (Parto et al., 2013; Sharifi et al., 2014) and red–leg syndrome (Parto et al., 2014), and, finally, a first trial re–introduction of captive–born newts (Sharifi and Vaissi, 2014). In addition, several laboratory studies have provided information on ontogenetic changes in spot configuration (Vaissi et al., 2017), cannibalism (Vaissi and Sharifi, 2016a), and growth and development of *N. microspilotus* in the captive breeding facility (Vaissi and Sharifi, 2016a, 2016b).

N. microspilotus in its natural environment acts as a top predator and feeds on a variety of prey, including benthic macroinvertebrates, amphibian eggs and tadpoles (Farasat and Sharifi, 2014), while the adult captive–born newts consume readily available food such as blood worm (*Glycera dibranchiate*), earthworm (*Lumbricus terrestris*) and chopped mealworm (*Tenebrio molitor*) *ad libitum*. The green toad, *Bufotes variabilis,* is a common toad in Iran with a wide distribution in most temperate areas, including parts of the distribution of the *N. microspilotus* in western Iran (Dastansara et al., 2017). We hypothesized that captive born *N. microspilotus* may lose their predatory ability to search, seize and engulf their natural prey (Burghardt, 2013). But we also postulated that captive born *N. microspilotus* could restore their predatory capability if they encountered natural prey. We therefore aimed to measure the predatory impact of captive–born adults at different densities and compare their impact with the free living newts under similar laboratory conditions in order: i) to determine whether there is a difference in the predatory impact of captive–born and free–living newts by examining the survival and growth rate of tadpoles of green toad (*Bufotes variabilis*) as prey, and ii) to measure whether captive newts can increase their predatory ability on tadpoles over time.

Material and methods

To experimentally assess the predatory ability of captive–born newts (CBN), we used eighteen adult individuals (6–year–old) with SVL (mean ± SD) of 64.37 ± 4.99 mm and body mass (mean ± SD) of 8.03 ± 1.40 g. The captive newt stock originated from two gravid females captured from Kavat stream (35º 21′ N, 46º 24′ E) on 5 February 2010. Experimental individuals were raised to maturity in aquaria boxes with electrical ventilators, waste pumps, and water temperature ranging from 15 to 21 ºC in summer and from 3 to 12 ºC in winter. Embryonic development (from oviposition to hatching) usually takes 28 d. Once hatched, larval newts were placed in separate aerated plastic containers (length x width x height: 22 × 16 × 12 cm) to prevent cannibalism, and they were fed by *Artemia* egg and shredded blood worms (*Glycera dibranchiate*). The larval period lasted six to eight months, until metamorphosis (loss of gills) was reached. Metamorphosed individuals were transferred to an aquarium (length x width x height: 49 × 21 × 16 cm) that contained small pebbles taken from their natural habitat to create approximately one third of their aquarium (300–350 cm^2) as a terrestrial habitat. Recently metamorphosed individuals left the water, mostly occupying the terrestrial portion of their aquaria. They were fed *ad libitum* with gradually larger food items (blood worms, earthworm, and live mealworms). Adults were kept together, numbering six to nine newts, in a single aquarium (length x width x height: 75 × 25 × 40 cm) filled with mosses and some aquatic plants, with a sex ratio of two males to one female. Eighteen wild–caught adult individuals (FLN): SVL (60.79 ± 5.04 mm) and body mass (7.59 ± 1.30 g), were collected from the same population (Kavat stream) on 14 May 2016 and brought into the laboratory. They were kept, for one week before testing, in an aerated aquarium containing dechlorinated tap water and allowed to acclimatize in the laboratory, in similar conditions to CBN individuals.

We collected three clutches of *B. variabilis* from Sarable wetland (34º 32' N, 47º 01' E), Kermanshah Province, Iran, in April 2016. Embryos were transferred to plastic containers until the experiments started, and they were allowed to develop and hatch in the laboratory in 12:12 light–dark cycles at approximately 20 ºC. Tadpoles were fed boiled spinach daily, and the water in all containers was changed weekly. The experiment was started when tadpoles were at the Gosner's developmental stages 25–26 (Gosner, 1960) and within the consumable size range for the predators. SVL and body mass at the start of the experiments were 1.33 ± 0.58 cm and 0.035 ± 0.004 g, n = 50. The surviving tadpoles and all eighteen FLN newts were released back to their original habitats after the experiments were finished. The study was conducted with the approval of the Razi University ethics committee under permit number: 3962022.

Our experimental design tests for two sources of variation: predator origin, involving two levels: CBN and FLN, and predator density, with three levels: low (L), medium (M), high (H) and a control (1, 2, 3 and 0 newts, respectively). This design determines eight different predator origin and density combinations (including controls) and was replicated three times, for a total of twenty–four containers. Each container (49 × 21 × 16 cm) was filled with 550 ml of dechlorinated tap water. Fifty larvae of green toad, *B. variabilis*, were added in each experimental container. Tadpoles and predators were visually matched for size so that the initial differences in size would be minimal. We started the experiment on 21 May 2016 and randomly selected tadpoles and the newts to be used in the twenty–four experimental containers of predator origin and predator density treatment combinations: CBF–H, CBF–M, CBF–L, FLN–H, FLN–M, FLN–L and controls. For each container, we added four similar size stones to provide spatial heterogeneity. All containers were covered with mosquito cloth mesh to prevent newts from crawling out of the containers. Predators were weighed and measured prior to the start of the experiments. During the twenty–five days of the experiment, containers were checked daily, and the number of surviving tadpoles in each container was recorded. At each observation, dead and partly eaten tadpoles were removed from the containers and absent tadpoles were considered as preyed. Every four days, tadpole body length was measured in each container. Photographs were taken with a digital camera (Sony, DSCHX9V) on a tripod at a fixed height (30 cm). The tadpoles were put in a bowl which was located over a graph paper. Immediately after photography, the tadpoles were released into their containers. All pictures were loaded into Digimizer 4 software and the tadpole total length (TL) was measured by drawing a line from the tip of the snout to the tip of the tail. The containers were cleaned and water was renewed with dechlorinated tap water every eight days. During the experiment, tadpoles were fed boiled spinach every day *ad libitum.*

All variables were checked for normality and homogeneity using the Kolmogorov–Smirnov normality test and Levene's test, respectively. We used two–way analysis of variance (ANOVA) to examine the effect of both predator origin and predator density on predation rate (the mean number of preyed tadpoles in each container during twenty–five days) and growth rate of tadpoles total body length (mm/day). Tukey's HSD post hoc pairwise comparisons were used to determine differences in predation rate between different newt densities. We compared the average number of preyed tadpoles in five–day periods for CBNs using repeated measure analysis of variance (repeated measure ANOVA) to determine whether predatory ability can improve with time. All statistical analyses were carried out using SPSS statistical software, version 16.0 (SPSS Inc, 2007). The statistical significance level was 0.05.

Results

The results of two–way ANOVA indicated a significant effect of newt origin and density and their interaction on the predation rate (table 1). In all densities of newts, the predation rates on tadpoles were higher in FLNs than in CBNs (table 2). High predator density greatly decreased tadpole survival, but this decrease was more drastic in FLN newts than in CBN newts, as revealed by the significant interaction term. Tukey's post hoc test showed that the highest predation rate was found for the FLN high density treatment. A repeated measure ANOVA at 5–day intervals showed that the predatory ability in CBNs, although lower than that of FLNs, increased with time ($F_{4,\ 32}$ = 4.38, P = 0.006). FLN predation rate was maximal (18.11 ± 13.91 eaten tadpoles) in the first interval but did not increase later (fig. 1B), whereas the predatory ability of CBNs was low at the beginning but increased over time (3.66 ± 2.50 eaten tadpoles in the first interval to 7.33 ± 4.30 eaten tadpoles in the third interval) (fig. 1A).

In addition, two–way ANOVA showed that the predator origin and predator density did not have a significant effect on the growth rate of the green toad, *B. variabilis* (table 1). However, there was a significant difference

Table 1. Results of univariate ANOVA for assessing effects of predatory origin (captive–born vs free–living) and density of newts (3, 2, and 1) on predation rate and prey growth rate.

Tabla 1. Resultados de la ANOVA univariada realizada para evaluar los efectos del origen del depredador (nacimiento en cautividad o en la naturaleza) y la densidad de los tritones (3, 2 y 1) en la tasa de depredación y la tasa de crecimiento de las presas.

	Source of variation		
	F	df	*P*–value
Predation rate			
Predator origin	49.24	1	0.000
Density	17.26	1	0.000
Predator origin × Density	11.46	2	0.002
Growth rate			
Predator origin	1.590	2	0.231
Density	0.294	2	0.751
Predator origin × Density	0.257	2	0.777

Table 2. Mean ± SD for predation rate (%), and growth rate (mm/day) on fifty larvae of green toad, *Bufotes variabilis* under different predator origin (CBN and FLN) and predator density (High, Medium and Low) during the twenty–five days of the experiment.

Tabla 2. Media ± DE de la tasa de depredación (%) y la tasa de crecimiento (mm/día) en 50 larvas de sapo verde, Bufotes variabilis, con depredadores de origen diferente (CBN y FLN) y distinta densidad de depredadores (alta, media y baja) durante los 25 días del experimento.

Treatment	Predation rate	Growth rate
FLN–H	78.54 ± 11.13	0.42 ± 0.33
FLN–M	40.29 ± 11.45	0.37 ± 0.10
FLN–L	26.32 ± 8.38	0.50 ± 0.10
CBN–H	22.77 ± 6.65	0.35 ± 0.05
CBN–M	17.01 ± 6.40	0.33 ± 0.06
CBN–L	18.10 ± 7.27	0.33 ± 0.09
Control	17.85 ± 7.02	0.24 ± 0.04

between the predator origin and the control on the growth rate of tadpoles (ANOVA, *P*–value = 0.037). Tukey's post hoc test indicated that free–living predators caused a significant increase in the growth rate compared with the controls (*P*–value = 0.03).

Discussion

Our results are in agreement with our expectations. First, we found that newts raised in captivity had lower predation rates than the free–living newts. In addition, we observed that the predation rate in CBNs improved when they were exposed to the green toad *B. variabilis* tadpoles during the short experimental period (25 days). This finding suggests the predatory abilities of the captive stock newts increased. As in accordance with out study design we did not replace the consumed prey to keep the original prey densities constant, we could expect lower predation rates simply by reducing the chance of prey encounter by newts. However, the observation that CBNs newts increased their tadpole consumption rate is probably explained by increased capacities in prey recognition and hunting abilities over time. These results indicate that husbandry conditions of captive born newts can improve predatory ability by initiating prey–predator interaction in the breeding facilities. Under natural conditions, *N. microspilotus* is an active predator moving through the water column and ground feeding on macroinvertebrates, eggs and tadpoles (Farasat and Sharifi, 2014). As a result, active movements in aquatic environments increase the chances of capturing prey (Hossie and Murray, 2010). In our study, CBNs were fed several food items, such as earthworms, bloodworms and mealworms *ad libitum,* possibly meeting optimal nutritional need. However, such an approach may cause inactivity and obesity (McWilliams, 2008). Therefore, the initial assumption of predation rates being higher among FLN newts was demonstrated, probably because the prey capture efficiency and foraging was lower in the newts of captive origin. By comparing both captive–born and free–living *N. microspilotus,* we found both groups were similar in performing stereotype behavior of approaching, seizing and sudden engulfing tadpoles. However, the newts reared in the captive environment differed in their boldness and speed of their attack and showed less success in capturing the tadpoles than their free–living conspecifics (T. Salehi and M. Sharifi, pers. observ.).This may explain their constrasting predatory rates.

Secondly, this study shows that exposing captive–born adult newts to a potential prey improve their predatory ability, suggesting that an environmental enrichment such as that used in this study, can be effective in developing more efficient captive breeding and reintroduction programs in *N. microspilotus.* Burghardt (2013) and Michaels et al. (2014) reviewed studies on how changes in different aspects of the environmental enrichment may improve the individual welfare of captive–born amphibian species. For instance, feeding enrichment in *Dendrobates tinctorius*, *D. azureus*, *D. auratus*, *D. leucomelas*, and *Oophaga pumilio* increases their activity levels and foraging behaviour (Campbell–Palmer et al., 2006). A study on Golden Mantella frogs (*Mantella aurantiaca*) showed that a diverse diet and a variety of live invertebrates, together with vitamins and mineral supplementations, can affect body condition and normal behaviour (Pas-

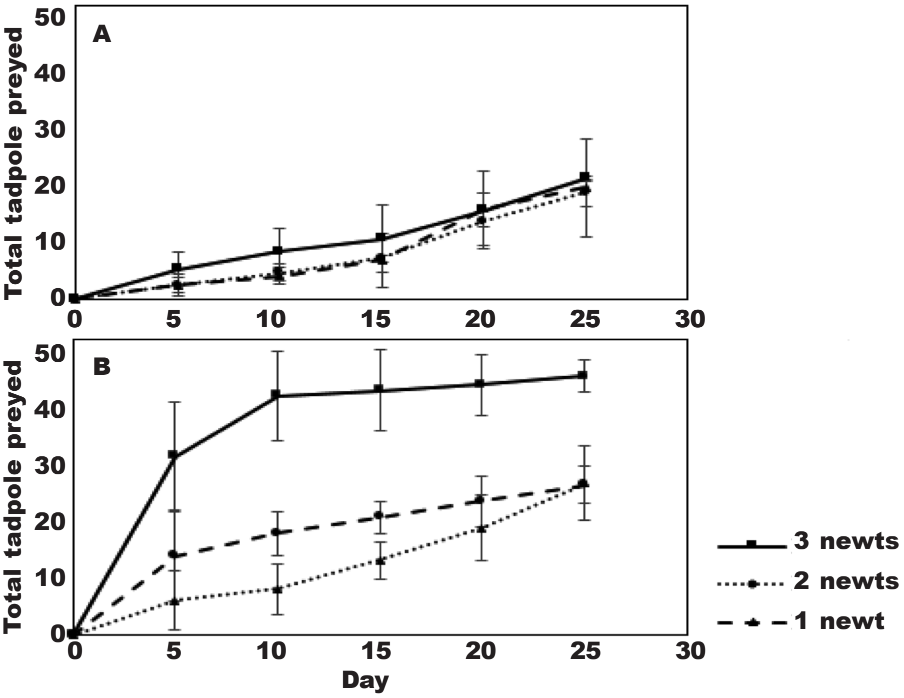

Fig. 1. Average and standard deviation of the predation rate (total number of tadpoles consumed in 5–day intervals: by captive–bred (A) and free–living (B) *Neurergus microspilotus* exposed to different treatments (H, 3 newts; M, 2 newts; L, 1 newt) over the twenty–five days of the experiment.

Fig. 1. Media y desviación estándar de la tasa de depredación (número total de renacuajos consumidos en intervalos de cinco días por individuos de Neurergus microspilotus criados en cautividad (A) y criados en libertad (B) expuestos a distintos tratamientos (H, 3 renacuajos; M, 2 renacuajos; L, 1 renacuajo) durante los 25 días del experimento).

sos et al., 2017). Similarly, enclosures supplemented with spatial heterogeneity can increase activity levels in captive *Lithobates catesbeianus* (Rose et al., 2014). In addition, to hide or scatter food items throughout captive rearing enclosures can encourage natural foraging behaviour and predatory ability (Poole and Grow, 2012). Summarizing determining best feeding regimes for captive–born species can provide opportunities to mimic the situation where these species may be introduced into the wild (Keulen and Janssens, 2017).

Interaction between predator origin and predator density influences tadpole predation rates. Predator density decreases prey survival in FLN newts but not in the captive reared newts. This will imply a much lower individual ingestion rate at higher densities in captive origin newts, which may have implications on net growth and breeding performance. A range of spatial organizations with increasing densities of amphibians and reptiles in the wild has been reported, but captive environment changes these organizations due to spatial constraints (Hayes et al., 1998). Thus, a lower ingestion rate in high density CBNs may help to choose lower raising densities in order to enhance growth rates and ultimately increase the viability of the captive stock.

The effects of the density of predatory newts on anuran larvae as prey are not always predictable. Morin (1986) has shown that relative abundance of larval anurans may be positively or negatively altered by densities of predatory salamanders in pond communities. Morin (1983) showed that relative abundances of prey tadpoles of some species (*Scaphiopus holbrooki*, *Rana sphenocephala*, *Bufo terrestris*, and *Hyla chrysoscelis*) at high predator salamander densities (*Notophthalmus viridescens dorsalis*) were reduced, whereas the relative abundances of *H. crucifer* and *H. gratiosa* tadpoles in the presence of newts were not affected. Various explanations have been proposed for the differences found in the intensities of salamander predation on amphibian tadpoles (Morin, 1986). Individual predators may influence one another's predation rate when foraging on prey (Ramos and Van Buskirk, 2012), or changes in prey behaviour (Charnov et al., 1976) which can reduce predator success at higher predator densities. Our results suggest that predatory ability on tadpoles increased with density in the FLN group, thus suggesting that individual predators did not affect each other. One possibility of larval salamander interference is cannibalism reported for larvae of *N. microspilotus* (Vaissi et al., 2017). However, neither cannibalism nor even apparent aggressive interactions occurred during our experiments.

Overall, predator origin and density did not influence the growth rate of surviving tadpoles. However, by comparing the growth rate of tadpoles between control and predator treatments, we observed that predators induced higher and significant growth rates in FLN newts. Current evidence suggests that the presence and origin of predators have a great influence on prey growth rates (e.g. McPeek et al., 2001; Relyea, 2004; Van Buskirk, 2009). Prey responses to predators are widespread among larval amphibians. Rapid growth, for example, may reduce or even avoid larval predation by becoming too large for predators (Brodie and Formanowicz, 1983). However, other evidence has indicated that the presence of the non–lethal predator may induce reductions in growth rate (Van Buskirk, 2002). The increase in tadpole size in the presence of predators may be an adaptive strategy of prey known as the 'gap limited predator', a mechanism that reduces predation intensity when a predator cannot consume larger tadpoles. Another possible explanation for the higher growth rate in prey species is 'thinning', that is, an indirect effect of predators by removing competitors, leading to an increase in the per capita food resources for surviving prey (Abrams and Rowe, 1996). Although tadpole survival decreased at high FLN newt density, we did not find an expected increase in growth rates at high density, suggesting that no thinning mechanism is occurring. A possible explanation for the absence of competition in tadpoles under high densities of newt predators could be a strong reduction in activity by surviving tadpoles that will reduce their ingestion rates and, therefore, exhibiting a decline in their growth rates (e.g. McPeek et al., 2001). In conclusion, the significant increase in growth rate of FLN tadpoles suggests that *N. microspilotus* may be a 'gap limited predator' able to reduce predation intensity when they cannot consume large tadpoles.

In conclusion, our results emphasize that exposing captive–born adult *N. microspilotus* newts to their potential prey can enrich the environment and may be a promising approach in the development of efficient captive breeding and reintroduction programs for this highly endangered amphibian.

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References

Abrams, P. A., Rowe, L., 1996. The effects of predation on the age and size of maturity of prey. *Evolution,* 50: 1052–1061.

Afroosheh, M., Akmali, V., Esmaelii, S., Sharif, M., 2016. On the distribution, abundance and conservation status of the endangered Spotted Mountain Newt *Neurergus microspilotus* (Caudata: Salamandridae) in Western Iran, *Herpetological Conservation and Biology*, 11: 52–60.

Armstrong, D. P., Seddon P. J., 2008. Directions in reintroduction biology. *Trends in Ecology and Evolution*, 23: 20–25.

Brodie Jr, E. D., Formanowicz Jr, D. R., 1983. Prey size preference of predators: differential vulnerability of larval anurans. *Herpetologica*, 39: 67–75.

Browne, R. K., Zippel, K., 2007. Reproduction and larval rearing of amphibians. *ILAR journal*, 48(3): 214–234.

Burghardt, G. M., 2013. Environmental enrichment and cognitive complexity in reptiles and amphibians: concepts, review, and implications for captive populations. *Applied Animal Behaviour Science*, 147(3): 286–298.

Campbell–Palmer, R., Macdonald, W. C., Waran, N., 2006. The effect of feeding enrichment on the behavior of captive Dendrobatid frogs. *Zoo Animal Nutrition,* 3: 315.

Charnov, E. L., Orians, G. H., Hyatt, K., 1976. Ecological implications of resource depression. *American Naturalist,* 110: 247–259.

Dastansara, N., Vaissi, S., Mosavi, J., Sharifi, M., 2017. Impacts of temperature on growth, development and survival of larval *Bufo (Pseudepidalea) viridis* (Amphibia: Anura): implications of climate change. *Zoology and Ecology*, 27(3–4): 228–234.

Densmore, C. L., Green, D. E., 2007. Diseases of amphibians. *ILAR journal*, 48: 235–254.

Farasat, H., Sharifi, M., 2014. Food habit of the endangered yellow–spotted newt *Neurergus microspilotus* (Caudata, Salamandridae) in Kavat Stream, western Iran. *Zoological Studies,* 53(1): 61.

Gosner, K. L., 1960. A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica,* 16(3): 183–190.

Griffiths, R. A., Pavajeau, L., 2008. Captive breeding, reintroduction, and the conservation of amphibians. *Conservation Biology,* 22(4): 852–861.

Harding, G., Griffiths, R. A., Pavajeau, L., 2016. Developments in amphibian captive breeding and reintroduction programs. *Conservation Biology,* 30(2): 340–349.

Hayes, M. P., Jennings, M. R., Mellen, J. D., 1998. Beyond mammals: environmental enrichment for amphibians and reptiles. In: *Second Nature: Environmental Enrichment for Captive Animals*: 205–235 (D. J. Shepherdson, J. D. Mellen, M. Hutchins, Eds.). Smithsonian Institution Press, Washington, D.C.

Hedrick, P. W., Fredrickson, R. J., 2008. Captive breeding and the reintroduction of Mexican and red wolves. *Molecular Ecology*, 17(1): 344–350.

Hossie, T. J., Murray, D. L., 2010. You can't run but you can hide: refuge use in frog tadpoles elicits density–dependent predation by dragonfly larvae. *Oecologia,* 163(2): 395–404.

Keulen, J., Janssens, G., 2017. Nutrition of captive amphibians. Master's Dissertation, Ghent University.

Kraaijeveld–Smit, F. J. L., Griffiths, R. A., Moore, R. D., Beebee, T. J. C., 2006. Captive breeding and the fitness of reintroduced species: a test of the responses to predators in a threatened amphibian. *Journal of Applied Ecology*, 43: 360–365.

McPeek, M. A., Grace, M., Richardson, J. M., 2001. Physiological and behavioral responses to predators shape the growth/predation risk trade-off in damselflies. *Ecology*, 82(6): 1535–1545.

McWilliams, D. A., 2008. Nutrition Recommendations for some Captive Amphibian Species (Anura and Caudata). *Canadian Association of Zoos and Aquariums Nutrition Advisory Research Group*, http://www.caza-narg.ca/ref/amphibian%20nutrition%20report%20CAZA%202008.pdf [Accessed on September 2018].

Michaels, C. J., Downie, J. R., Campbell–Palmer, R., 2014. The importance of enrichment for advancing amphibian welfare and conservation goals. *Amphibian Reptile Conservation*, 8: 7–23.

Morin, P. J., 1983. Predation, competition, and the composition of larval anuran guilds. *Ecological Monographs,* 53(2): 119–138.

– 1986. Interactions between intraspecific competition and predation in an amphibian predator-prey system. *Ecology,* 67(3): 713–720.

Ogilvy. V., Preziosi, R. F., Fidgett, A. L., 2012. *A brighter future for frogs? The influence of carotenoids on the health, development and reproductive success of the red–eyed tree frog*. *Animal Conservation,* 16: 480–488.

Olfert, E. D., Cross B. M., McWilliam, A. A. Eds., 1993. *Guide to the care and use of experimental animals*, Vol. 1: 51–74 and 115–124. Canadian Council on Animal Care, Ottawa.

Parto, P., Siavash Haghighi, Z. M., Vaissi, S., Sharifi M., 2014. Microbiological and histological examinations in endangered *Neurergus kaiseri* tissues displaying Red–leg syndrome. *Asian Herpetological Research*, 5(3): 204–208.

Parto, P., Vaissi, S., Farasat, H., Sharifi, M., 2013. First Report of Chytridiomycosis (*Batrachochytrium dendrobatidis*) in Endangered *Neurergus microspilotus* (Caudata: Salamandridae) in Western Iran. *Global Veterinaria*, 11(5): 547–551.

Passos, L. F., Garcia, G., Young, R. J., 2017. The tonic immobility test: Do wild and captive golden mantella frogs (*Mantella aurantiaca*) have the same response? *Plos One*, 12(7): e0181972, Doi: 10.1371/journal.pone.0181972

Poole, V. A., Grow, S., 2012. Amphibian husbandry resource guide. Edition 2.0. *Association* of *Zoos* and *Aquariums*, Silver Spring, MD.

Pough, F. H., 2007. Amphibian biology and husbandry. *ILAR journal*, 48(3): 203–213.

Rabin, L. A., 2003. Maintaining behavioural diversity in captivity for conservation: natural behavior management. *Animal Welfare,* 12(1): 85–94.

Ramos, O., Van Buskirk, J., 2012. Non–interactive multiple predator effects on tadpole survival. *Oecologia,* 169(2): 535–539.

Relyea, R. A., 2004. Fine–tuned phenotypes: tadpole plasticity under 16 combinations of predators and competitors. *Ecology*, 85(1): 172–179.

Rose, P., Evans, C., Coffin, R., Miller, R., Nash, S., 2014. Using student–centred research to evidence–base exhibition of reptiles and amphibians: three species–specific case studies. *Journal of Zoo and Aquarium Research*, 2(1): 25.

Sharifi, M., Afroosheh, M., 2014. Studying migratory activity and home range of adult *Neurergus microspilotus* (Nesterov, 1916) in the Kavat Stream, western Iran, using photographic identification. *Herpetozoa*, 27(1/2): 77–82.

Sharifi, M., Bafti, S. S., Papenfuss, T., Anderson, S., Kuzmin, S., Rastegar–Pouyani, N., 2009. *Neurergus microspilotus*. In: *IUCN 2016: IUCN Red List of Threatened Species*, version 2016. 3, www.iucnredlist.org [Accessed on 07 December 2016].

Sharifi, M., Farasat, H., Vaissi, S., Parto, P., Siavosh Haghighi, Z. M., 2014. Prevalence of the Amphibian Pathogen *Batrachochytrium dendrobatidis* in Endangered *Neurergus microspilotus* (Caudata: Salamandridae) in Kavat Stream, Western Iran. *Global Veterinaria*, 12(1): 45–52.

Sharifi, M., Vaissi, S., 2014. Captive breeding and trial reintroduction of the endangered yellow–spotted mountain newt *Neurergus microspilotus* in western Iran. *Endangered Species Research,* 23: 159–166.

SPSS Inc., 2007. *SPSS for Windows*, Version 16.0. Chicago, SPSS Inc.

Stuart, S. N., Chanson, J. S., Cox, N. A., Young, B. E., Rodrigues, A. S., Fischman, D. L., Waller, R. W., 2004. Status and trends of amphibian declines and extinctions worldwide. *Science*, 306(5702): 1783–1786.

Vaissi, S., Sharifi, M., 2015. Larval and Post–Metamorphic Growth in the Endangered Yellow Spotted Mountain Newt *Neurergus microspilotus* (Caudata, Salamandridae). *World Journal of Zoology*, 10(4): 365–373.

– 2016a. Variation in food availability mediate the impact of density on cannibalism, growth, and survival in larval Yellow Spotted Newts (*Neurergus microspilotus*: implications for captive breeding programs. *Zoo Biology*, 35: 513–521.

– 2016b. Changes in food availability mediate the effects of temperature on growth, metamorphosis and survival in endangered Yellow Spotted Mountain Newts: implications for captive breeding programs. *Biologia,* 71: 444–451.

Vaissi, S., Parto, P., Sharifi, M., 2017. Ontogenetic changes in spot configuration (numbers, circularity, size and asymmetry) and lateral line in *Neurergus microspilotus* (Caudata: Salamandridae). *Acta Zoologica,* 99(1): 9–19.

Van Buskirk, J., 2002. A comparative test of the adaptive plasticity hypothesis: relationships between habitat and phenotype in anuran larvae. *The American Naturalist*, 160(1): 87–102.

– 2009. Natural variation in morphology of larval amphibians: phenotypic plasticity in nature? *Ecological Monograph*, 79: 681–705.

Zippel, K., Johnson, K., Gagliardo, R., Gibson, R., McFadden, M., Browne, R., Martinez, C., Townsend, E., 2011. The Amphibian Ark: a global community for ex situ conservation of amphibians. *Herpetological Conservation and Biology*, 6(3): 340–352.