PROCEEDINGS OF THE NATIONAL INSTITUTE OF ECOLOGY OF THE REPUBLIC OF KOREA

Original Articles

PNIE 2021;2(2):114-119
https://doi.org/10.22920/PNIE.2021.2.2.114
pISSN 2765-2203, eISSN 2765-2211

Post-release Monitoring after Reintroduction of Captive-reared Korean Endangered Frog, *Pelophylax Chosenicus*

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ABSTRACT

To restore the Gold-spotted pond frog (*Pelophylax chosenicus*), a Korean endangered frog, 600 captive-reared individuals were reintroduced between August and September 2019 into an aquatic garden in the National Institute of Ecology where *P. chosenicus* had previously inhabited. After reintroduction, six post-release monitoring sessions were conducted from August 2019 to May 2020. Monitoring was performed using three methods (counting calls, observation, and capture) from sunset to midnight near release sites, eliminating potential threat factors. Snout-vent lengths and body weights of recaptured individuals were measured before they were released immediately. We noted that both snout-vent lengths and body weights of recaptured individuals were slightly higher than those before. The average recapture rate was 4.66%. Reintroduced frogs were recaptured at the last monitoring session conducted in May 2020, indicating that these reintroduced frogs hibernated during the winter successfully. We found that these reintroduced frogs successfully settled after release. This result will be useful for establishing management strategies for endangered frogs in Korea. Particularly, post-release monitoring could be an essential approach in the restoration program of a target species.

Keywords: Gold-spotted pond frog, *Pelophylax chosenicus*, Post-release monitoring, Reintroduction, Restoration

Introduction

Amphibians are the most abundant vertebrates in wetland ecosystems, although they are relatively inconspicuous animals because of their small sizes, nocturnal activities, and preferred habitats in the ground or under rocks (deMaynadier & Hunter, 1995). During ontogenetic development, tadpoles and adults occupy two distinct habitats after metamorphosis. Therefore, habitat loss and fragmentation are fatal to amphibians moving between aquatic and terrestrial ecosystems. These are the most critical reasons why amphibian populations are declining worldwide (Becker *et al*., 2007).

Received September 10, 2020; **Revised** April 7, 2021; **Accepted** April 8, 2021

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A restoration program consists of three stages: stage 1, preparation; stage 2, implementation, and stage 3, post-restoration. In the preparation stage, the appropriateness of the restoration project is evaluated, the project is decided, and items for the project are prepared. The implementation stage is the actual implementation of the project. Finally, the post-restoration stage is conducted with post-restoration monitoring, evaluation, and public relations promotion (Gascon *et al*., 2007; International Union for Conservation of Nature (IUCN), Species Survival Commission (SSC), 2013; Ra, 2010). A monitoring period longer than 5 years is required to adequately assess site restoration regarding demographic responses due to amphibian characteristics such as significant population lags and sensitivity to site disturbances (Petranka *et al*., 2003).

Global amphibian populations have sharply decreased (IUCN, 2020). Major threat factors are habitat degradation, diseases, exotic species, pollution, and over-exploitation (Sung *et al*., 2009). *Pelophylax chosenicus* is designated as an endangered species by the Ministry of Environment, Korea. It is categorized as vulnerable by the IUCN. National Institute of Biological Resources (NIBR) (2018) has revealed that this species is also gradually decreasing for similar reasons such as habitat loss and destruction due to civilization, road construction, water quality degradation, pesticides, and exotic bullfrogs. Studies on *P. chosenicus* have been actively conducted in various fields, such as food sources (Yoon *et al*., 1998), habitats and behaviors (Park *et al*., 2018; Ra, 2010; Sung *et al*., 2009), genetic diversity (Park *et al*., 2009), and water quality (Borzée *et al*, 2018). However, no study related to restoration has been reported to date.

Reintroduction has been increasingly selected as one restoration method (Fischer & Lindenmayer, 2000; Griffith *et al*., 1989; Wolf *et al*., 1996). In several species with a limited ability to move like amphibians, reintroduction is required to repopulate sites where extirpation has already occurred (Blaustein *et al*., 1994). Post-release monitoring is an important stage in the restoration of a target species. Using monitoring data, we can estimate, modify, and complement a restoration program. Monitoring the current status of a reintroduced population is needed to demonstrate restoration success.

In this study, we conducted a reintroduction program for captive-reared *P. chosenicus*. After reintroduction, we performed six post-release monitoring sessions to investigate its successful settlement and estimate applicability of the reintroduction method to restore frogs in Korea.

Materials and Methods

Captive-breeding of *P. chosenicus*

P. chosenicus is designated as endangered species class II by the Ministry of Environment, Korea. We obtained permission from a regional environment office by law (Geum River Basin Environment Office, 2018-33). A total of 20 (male to female ratio = 1:1, classified by eye size vs. eardrum size) adults were collected from Asan City in September 2018 and transferred to the Endangered Species Restoration Center of the National Institute of Ecology (NIE). Collected frogs were reared in two PVC aquaria (99.0 cm in length× 45.0 cm in width × 40.0 cm in height). In these aquaria, a proper artificial habitat was set to rear *P. chosenicus*. A 10 cm depth peat moss mound with a sponge was provided to make a small land. The water depth was maintained at 5 cm. The water temperature was maintained at 27.0 ± 1.0°C using a titanium heater (OKE-HE185, Sewon, Korea) and a temperature regulator (OKE-6428HC, Sewon, Korea). The air temperature was regulated at 23.0 ± 1.0°C using an air temperature controller. During the daytime, additional light was supplied using a spot lamp to prevent light shortage. Adult crickets were provided as food once daily (two crickets per frog). During January 2019, captive-reared frogs spawned naturally in these aquaria [Certification of artificial breeding (Daegu Regional Environment Office, 2019-04)].

Selection of release site and reintroduction

The release site was selected by site observations and expert consultation. Among five candidate sites (e.g., paddy fields in Asan, Asan ecological environment insect museum, Duung wetland in Taean, wetland near the subway station in Ansan, and NIE), two sites (Asan ecological environment insect museum and the NIE) were selected as candidates for reintroduction. Finally, the NIE was selected as the best release site considering habitat conditions, threat factors, management, and SWOT analysis (Fig. 1). The release site, an aquatic garden, was in the NIE. This site had many advantages for managing our frog restoration program. Additionally, *P. chosenicus* was once inhabited the release site. Therefore, this site was ideal for reintroduction.

Post-release monitoring

Releases were conducted three times (August 19, August 29, and September 18) on the aquatic garden in the NIE. A total of 600 individuals were released (200 individuals per release). After reintroduction, post-release monitoring was conducted six times from August 2019 to May 2020 (1st: August 29; 2nd: September 4; 3rd: September 18; 4th: October 7; 5th: November 5, 2019; 6th: May 7, 2020). Monitoring was performed by counting calls, observation, and capture during sunset to midnight near the release site with elimination of potential threat factors. After measuring snout-vent length (SVL) and body weight (BW) of each recaptured individual, these individuals were then immediately released. Condition factor (CF) was calculated to estimate the nutritional status of *P. chosenicus* (Froese, 2006; Zhelev *et al*., 2017) using the following formula:

$$\text{Condition Factor} = (BW/SVL^3) \times 102$$

Statistical analyses

Linear regression analysis was conducted to identify growth patterns of recaptured individuals. Monitoring date was converted to Julian date before the calculation. To investigate size differences of captured frogs, Kruskal-Wallis tests were conducted. All statistical analyses were performed using Excel 2013 (Microsoft Corperation, USA) and R 4.0.2 (R Core Team, 2020).

Results and Discussion

Recapture and dispersion

We recaptured 27 individuals (recapture rate = 13.5%) among 200 individuals released during the 1st monitoring.

During the 2nd and the 3rd monitoring, 39 (9.75%) and 24 (6.0%) individuals were recaptured of 400 released individuals, respectively. During the 4th, 5th, and 6th monitoring, 46 (7.7%), 7 (1.2%), and 25 (4.2%) individuals were recaptured among 600 released individuals, respectively (Table 1). The average recapture rate was 4.66%, which was higher than that reported previously (Cheongwon: 0.5%, Taean: 0.3%; Ministry of Environment, Kangwon National University-Industry Cooperation Foundation, 2009).

Table 1. Comparison of recapture rate of *Pelophylax chosenicus* in this study and references

	This study							Reference	
	1st	2nd	3rd	4th	5th	6th	Total (average)	Cheongwon-gun (abandoned Paddy Field)	Taean-gun (duung-wetland)
Number of release/ recapture	200/ 27	400/ 39	400/ 24	600/ 46	600/ 7	600/ 25	600/ 28	700/4	300/1
Recapture rate (%)	13.5	9.75	6.0	7.7	1.2	4.2	4.66	0.57	0.33

The aquatic garden of the NIE is a well-managed place for reintroduction. However, several American bullfrogs (*Lithobates catesbeianus*), a predator of *P. chosenicus*, were present. Therefore, bullfrogs were continuously removed to stabilize the released *P. chosenicus* population during the entire monitoring period. A total of 592 bullfrog tadpoles and adults were removed from the 1st monitoring to the 6th monitoring. Additionally, one large individual of a largemouth bass (*Micropterus salmoides*) was captured and removed during the monitoring period.

Reintroduced *P. chosenicus* individuals were widely dispersed in aquatic gardens. The first reintroduced individuals released in the amphibian wetland moved to the aquatic garden. No individual was observed in the amphibian wetland. The aquatic garden is wide and filled with many aquatic plants, which are considerably useful as covers of frogs (Fig. 1).

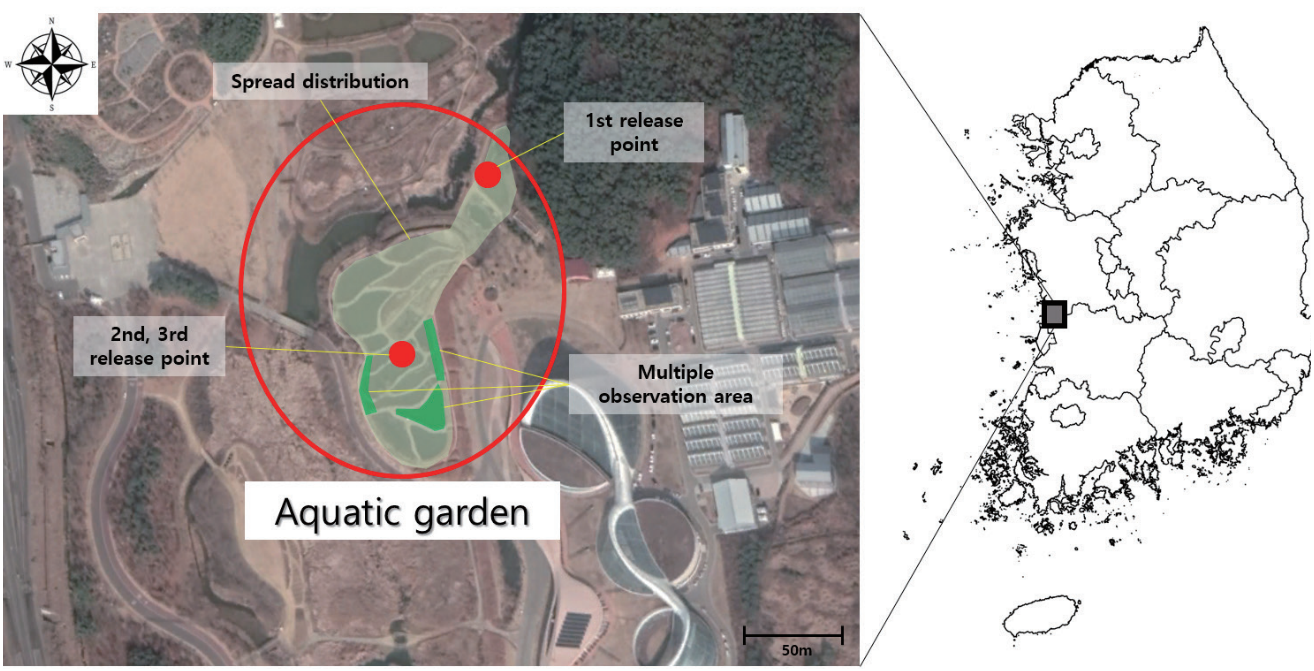

Fig. 1. Maps of aquatic garden in National Institute of Ecology

Body size changes of recaptured individuals

Both SVL and BW of recaptured individuals increased slightly during the monitoring period (Fig. 2). However, there was no significant difference in SVL (Kruskal-Wallis test, X^2 = 125.66, P = 0.32), BW (X^2 = 120.33, P = 0.28), or CF (X^2 = 126.04, P = 0.38) according to the order of monitoring (Table 2). Hibernation has evolved in many amphibians as a protective mechanism in response to cold temperatures, playing a prominent role in amphibian life-history strategies to survive under adverse environmental conditions (Pinde *et al*., 1992; Wells, 2007). Temperature is a major environmental factor that determines the hibernation period. Sex and age are key factors in phonological responses of *P. nigromaculatus*. Particularly, males emerge from hibernation earlier than females and old ones enter and emerge from hibernation earlier than young ones (Gao *et al*., 2015). Willis *et al*. (1956) have also demonstrated that larger individuals of *L. catesbe* begin hibernating earlier than smaller individuals. In contrast to the above studies, we found that *P. chosenicus* had greater SVL and BW at the 5th monitoring session (November 5, hibernating phase) than those at the 4th session (October 5) (Fig. 3). This meant that larger individuals of *P. chosenicus* began to hibernate later than smaller individuals. This result might indicate that older *P. chosenicus* were more adaptable than smaller individuals at the NIE in lower environmental conditions. They may feed to actively accumulate energy before entering hibernation. Future further release and monitoring require individual identification using VIE tags, PIT tags, and others. After the hibernating phase, sub-adults of *P. chosenicus* reintroduced in 2019 were detected on the 6th morning (May 2020). This finding indicated that reintroduced *P. chosenicus* individuals had successfully hibernated.

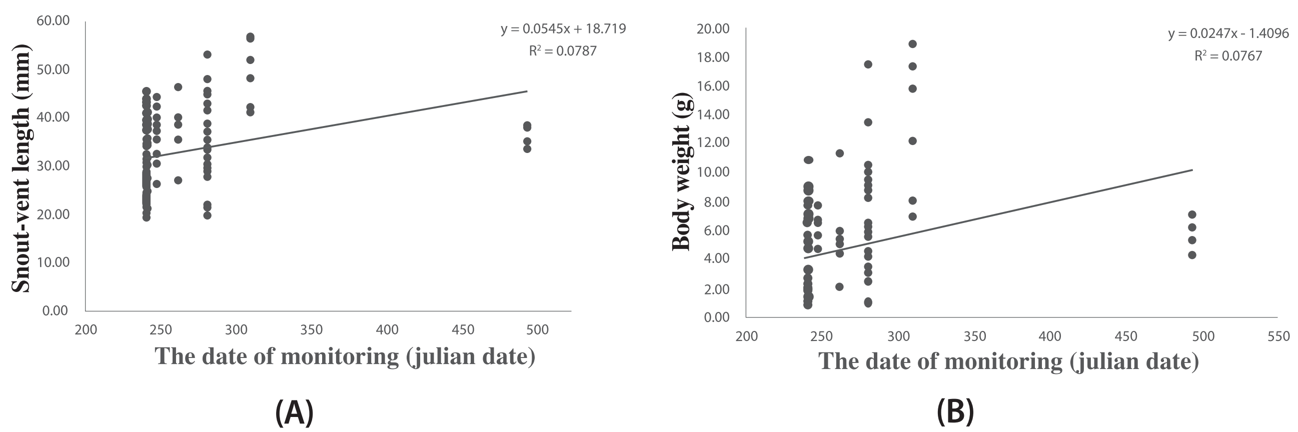

Fig. 2. Relationships between date of monitoring (Julian date) and (A) Snout-vent length (SVL), (B) Bodyweight (BW) of *Pelophylax chosenicus* on aquatic garden in National Institute of Ecology.

Table 2. Mean (± SD) snout-vent length (SVL), body weight (BW), and condition factor (CF) of *Pelophylax chosenicus* on aquatic garden in National Institute of Ecology according to monitoring order.

	Monitoring						X^2	P
	1st	2nd	3rd	4th	5th	6th		
SVL	35.79±6.77	35.90±5.74	37.61±5.84	36.21±8.12	49.50±6.72	36.34±2.28	125.66	0.32
BW	5.92±3.03	5.03±2.18	5.67±2.79	6.36±3.92	13.28±4.96	5.79±1.23	120.33	0.28
CF	12.93±2.72	10.87±1.33	10.66±1.17	13.39±0.91	10.95±0.86	12.05±1.01	126.04	0.38

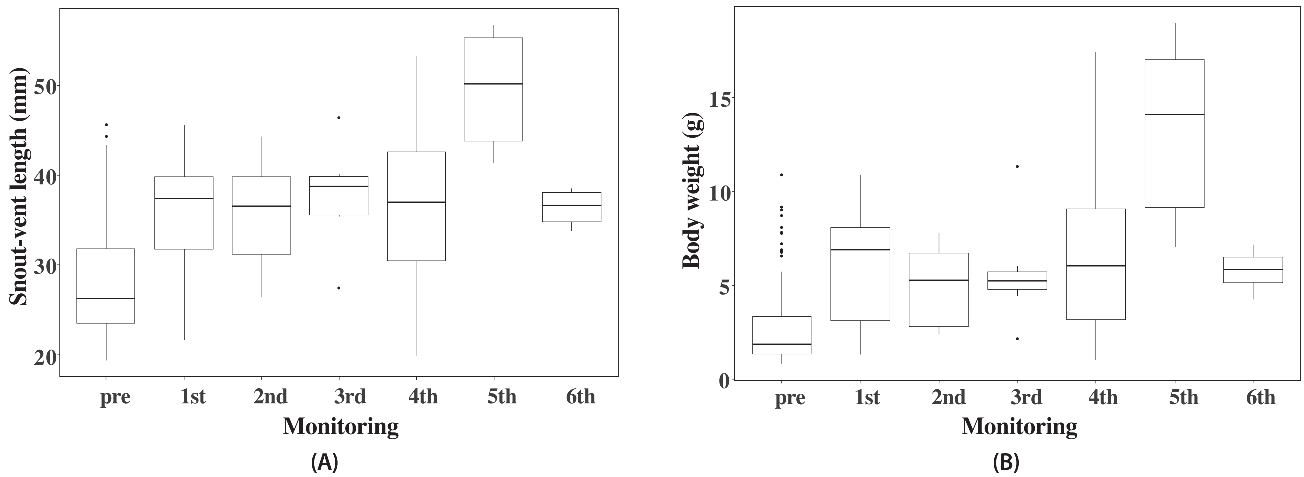

Fig. 3. Size distributions of *Pelophylax chosenicus* on aquatic garden in National Institute of Ecology ((A) Snout-vent length (SVL), (B) Body weight (BW)) by date of monitoring.

There are two levels of indicators of restoration success. One is an early indicator related to the survival of eggs and larvae through metamorphosis, recruitment of juveniles into adults, and breeding activity. The breeding activity was demonstrated by egg-carrying females, calling males, amplexus, and spawning. The other is a long-term indicator related to the establishment of adult population size (excess of 50 individuals within 5-10 years), mixed population structure (with juveniles and adults regularly recorded, and regular breeding success), and progressive colonization into multiple ponds (establishment of a robust meta-population structure at least five over 5-10 years) (Baker & Foster, 2015).

P. chosenicus takes at least 2-3 years to mature sexually (NIE, 2018). In a future study, sexual maturity of *P. chosenicus* reintroduced in 2019 should be observed by monitoring their eggs and tadpoles through their breeding activities. A normal growth of their offspring in the NIE would indicate the success of restoration by satisfying the early indicator. Annual monitoring results should be assessed against the success criteria described above as restoration projects develop. Post-reintroduction monitoring periods commonly last 3 and 5 years following mitigation of the restoration of a target species (National Research Council, 2001).

To achieve restoration success of *P. chosenicus*, continuous monitoring is required for at least 3 years. Therefore, more post-release monitoring of *P. chosenicus* should be conducted in the aquatic garden of the NIE. Findings of the current study provide fundamental information for restoration and conservation strategies throughout post-release monitoring after reintroduction of *P. chosenicus.*

Conflict of Interest

The authors declare that they have no competing interests.

Acknowledgments

This work was supported by a grant (NIE-B-2021-49) from the National Institute of Ecology (NIE) funded by the Ministry of Environment (MOE), Republic of Korea. interests.

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