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Population Dynamics of the Long-Tailed Clawed Salamander Larva, *Onychodactylus fischeri*, and Its Age Structure in Korea

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ABSTRACT: Larvae of the long-tailed clawed salamander, *Onychodactylus fischeri*, have a relatively long larval period, spending a year or more within the stream where they hatch; therefore, a well-established larval population could be critical for the conservation of adult populations. To study the population dynamics of long-tailed clawed salamander larvae, we surveyed a field population once or twice a month from September, 2005 to June, 2006, and determined the age of larval clawed salamanders collected from three different populations in October, 2004 using skeletochronology. The age of long-tailed clawed salamander larvae ranged from 0 to 3 years. New recruitment of larvae in the population primarily occurred in November, 2005, and mid-March, 2006. Larvae with a snout-vent length of more than 30 mm disappeared from the streams in September, 2005, suggesting that two to three year-old clawed salamander larvae metamorphosed during this period.

Key words: Breeding season, Long-tailed clawed salamander, Metamorphosis, *Onychodactylus fischeri*, Skeletochronology

INTRODUCTION

Salamanders in Hynobiidae are considered to be the most primitive urodele group (Salthe 1967, Zhang et al. 2006). Unlike most other urodeles, which internally fertilize eggs, they use external fertilization (Salthe 1967). The clawed salamanders, *Onychodactylus*, are characterized by unique claws on the tips of their digits and consist of two different species: the Japanese clawed salamander, *O. japonicus*, which is native to Japan, and the long-tailed clawed salamander, *O. fischeri*, which is found mainly on the Korean peninsula (Kuzmin 1995). Several small peripheral populations of this species are also situated in Russia and China (Griffin and Solkin 1995, Kuzmin 1995, Maslova 1998). Compared to European newts and Asiatic *Hynobius* salamanders for which general and reproductive ecology have been relatively well-studied (Houck and Arnold 2003), only a few studies of the ecology and reproduction of the clawed salamander have been conducted.

Due to the fact that, in Korea, the long-tailed clawed salamander breeds in well-reserved mountain streams where water temperature is relatively low and freshwater fishes do not disrupt their breeding and larval growth, the species has been considered as a representative indicator of the health of the mountain stream system. Depending on the area and populations studied, long-tailed clawed salamanders may breed once or twice a year under a large rock in the streams or under the ground where light does not penetrate (Kang and Yoon 1975, Park 2005). Oviposition occurs between June and July, and during egg fertilization, strong male-male mating competition has been observed, resulting in the formation of a mating ball (Park 2005). In laboratory experiments and field observation, fertilized eggs hatch approximately 5 months after fertilization (Iwasawa and Kera 1980, Personal observation). Unlike *Hynobius* species whose larvae metamorphose about 3 moths after hatching, larvae of the clawed salamander have a relatively long larval period of a year or more in the native stream where they were hatched (Hayase and Yamane 1982, Kozik and Truberg 1985). Thus, conserving larval populations in the mountain streams is a critical step in conserving healthy adult populations of clawed salamanders in the field. Still, very few studies have addressed when larval salamanders appear in the population, how long they remain in the stream, and when they metamorphose (Regel and Epstein 1975, Solkin 1993). Furthermore, these studies were all done in Russian or Japanese clawed salamander populations. Only one study has been done previously in a Korean population (Park 2005).

To study the population dynamics of long-tailed clawed salamander larvae in Korea, we surveyed a field population once or twice a month from September, 2005 to June, 2006, and determined the age of larval salamanders collected from three different populations in October, 2004 using skeletochronology.

MATERIALS AND METHODS

To study the dynamics of clawed salamander larvae in a field population, we surveyed the population once or twice a month from

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September 4, 2005 to June 18, 2006. The population was within a small mountain stream located at Goeon-Ri, Dongnae-Myeon, Chuncheon-Shi, Kangwon-Do, South Korea. We selected two survey sites, each approximately 2 m wide × 2 m long × 10 cm deep. During a survey, we captured as many larval salamanders within the site as possible using a hand net and anesthetized them with 0.05% MS222 (3-aminobenzoic acid ethyl ester, Sigma) for 5 min. We measured their snout-vent length (SVL) to the nearest 0.1 cm using a digital caliper (Mitutoyo; CD-15CPX) and measured body weight to the nearest 0.1 g using a digital field balance (CB-1200). After measuring these physical parameters, we released all of the larvae at the same site where we captured them. We confirmed that the anesthetized larvae had recovered within 10 min and began activities.

To determine the age of clawed salamander larvae, we used a hand net to collect 33, 39, and 30 larvae from Goeon-Ri in Dongnae-Myeon, Dongsan-Ri in Jinbu-Myeon, and Myeongji-Ri in Nae-Myeon, Kangwon-Do, South Korea, respectively. To collect salamander larvae, we first designated a 1 m × 1 m area within a stream where we found many larval salamanders and collected as many larvae as possible in the area using a hand net. At the collection site, we sacrificed the larval salamanders with over-exposure to 0.1% MS222 and measured their physical parameters including total length, SVL, head length, head width, tail depth, and body weight to the nearest 0.1 cm or 0.1 g. Head length was measured from the tip of snout to the head line, and tail depth was defined as the largest width of the tail. After measuring those parameters, we fixed the larvae in 10% neutral formalin (pH 7.6) for subsequent skeletochronological study. Since we needed to have specimens of clawed salamander larvae, we fixed all individuals instead of cutting the digits of each individual.

In the laboratory, we clipped two digits from the phalanges of each reserved larval salamander. To prepare the clipped toes for skeletochronology, we followed the procedures given by Hemelaar and van Gelder (1980). In brief, we first cleaned the clipped toes in the laboratory by washing them in running tap water for 24 hrs and decalcified them by submerging the toes in 5% nitric acid for 20～30 min. After another 24 hrs of washing with tap water, we paraffin-embedded the toes after a serial dehydration processes. A rotary microtome (Erma Inc. Tokyo) was used to cut 10 μm thick sections of each toe. Cut sections were stained according to the Harris Eosin-Haematoxylin method (Presnell and Schreibman 1997) and observed under the microscope (× 400). Growth zones and LAGs (line of arrested growth) were visible in cross sections of the phalanges (Fig. 1). The number of LAGs was counted according to Leary et al (2005), and individual ages were independently determined by two authors. When the determinations of the two authors were different, we corroborated scores of the LAGs and drew a consensus conclusion.

Since body weight and SVL of clawed salamander larvae both showed similar dynamics during the study period, we only present the frequency distribution of the SVL over that period. To determine whether or not three field populations had different age structures, we used the Chi-squared test. For the comparison of physical parameters of clawed salamander larvae among three populations, we only used data from 73 individuals for which age was determined, out of a total of 112 individuals. Since the data did not meet the normality assumption for a parametric analysis (Kolmogorov-Smirnov, $P > 0.05$), we used the Kruskal-Wallis test to determine if the total length, SVL, head length, head width, tail width and body

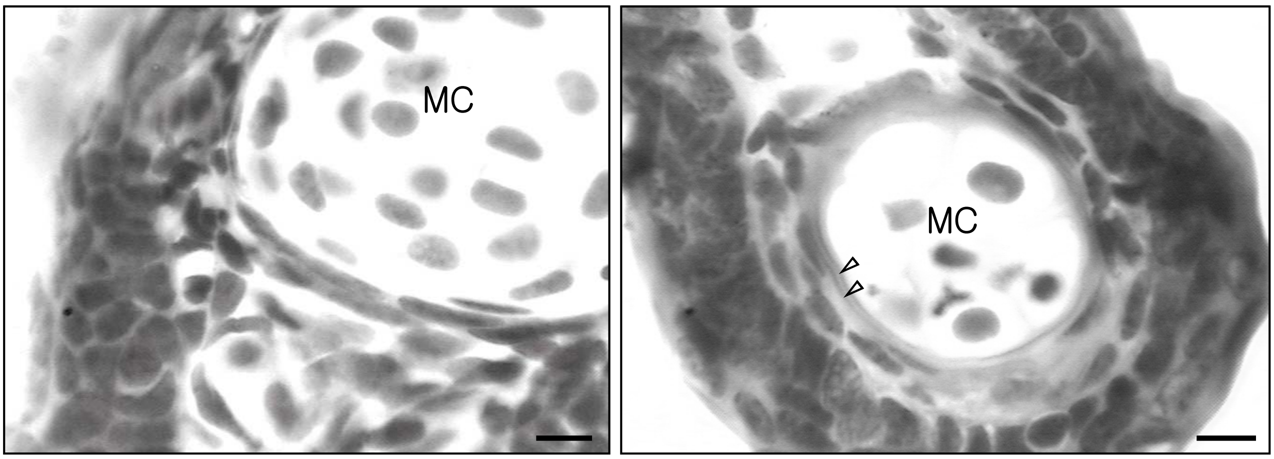

Fig. 1. Phalangeal cross sections of a 0-year-old (left) and a 2-year-old (right) long-tailed salamander larva, *Onychodactylus fischeri*, from the Goeon-Ri population. Arrows in the right picture indicate the line of arrested growth (LAG). No LAG was present in the phalangeal section of the 0-year-old salamander larva. MC represents the medullary cavity. Bars 30 μm.

weight were different among the populations, followed by the *post-hoc* test (Siegel and Castellan, 1988) if the test was significant. All statistical analyses were two-tailed and performed using SPSS version 11.0 (SPSS Inc., Chicago Illinois, USA). All values were reported as mean ± SE. This study was approved by the Korean government. All experimental procedures followed the Guidelines for use of live amphibians and reptiles in field and laboratory research (ASIH 2004).

RESULTS

From the population survey, we observed a total of 338 clawed salamander larvae over a 10-month period (Fig. 2). In March, 2006, we observed the most individuals (59 larvae), and in December, 2005, we observed the fewest individuals (20 larvae). The SVL of the larval salamanders ranged from 17 to 44 mm. The number of individuals with an SVL larger than 30 mm was decreased in September, 2005. In mid-November, 2005 and mid-March, 2006, many individuals with an SVL of less than 24 mm were newly present in the population. Over the survey period, individuals with an SVL larger than 38 mm were always present, although their numbers varied.

We successfully determined the ages of 73 clawed salamander larvae out of 102 total larvae. The age of the larval salamanders

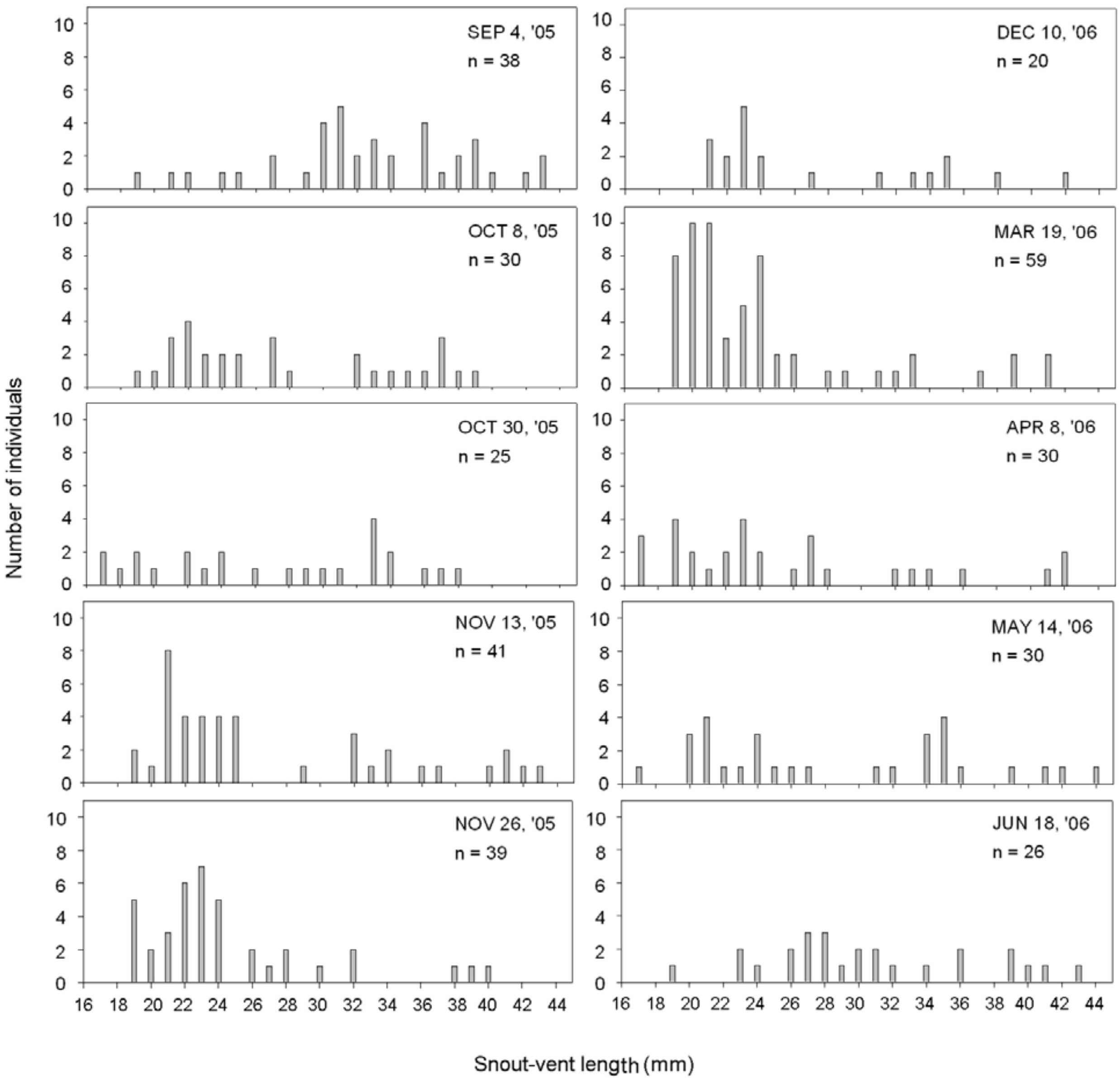

Fig. 2. Size frequency distribution of long-tailed clawed salamander larvae, *Onychodactylus fischeri*, within a Goeon-Ri population over 10 months.

varied significantly from 0 to 3 years old (Chi-square = 32.70, *df* = 3, $P < 0.001$). More one- (20 larvae) and two-year-olds (37 larvae) were found than 0- (4 larvae) and 3-year-olds (12 larvae). The age structures of the three populations were different (Chi-square = 20.294, *df* = 6, $P = 0.0025$, Fig. 3). Specifically, the Goeon-Ri population had more 2-year-old larvae than the other populations (Chi-square test, $P < 0.05$). Based on the different ages of larval salamanders, differences in total length, SVL, head length, head width, tail width, and body weight were significant (Kruskal-Wallis test, $P < 0.05$ for the cases, Table 1). For each parameter, the values for 2- and 3-year-old larvae were larger than those for 0- and 1-year-old larvae (*Post-hoc* test, $P < 0.05$ for the cases), while each parametric value between 0- and 1-year-olds and between 2- and 3-year-olds was not different (*Post-hoc* test, $P > 0.05$ for the cases).

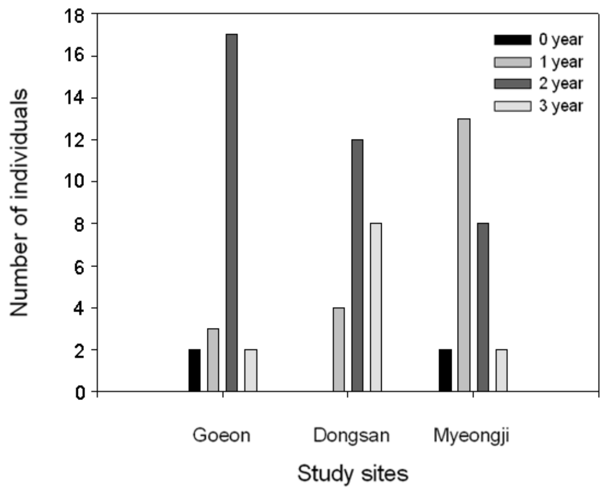

Fig. 3. Age distribution of long-tailed clawed salamander larvae, *Onychodactylus fischeri*, in the Goeon-Ri, Dongsan-Ri, and Myeongji- Ri populations.

DISCUSSION

To study the population dynamics of long-tailed clawed salamander larvae, we surveyed a field population of the species over 10 months and determined the ages of 73 individuals caught from three populations. The age of larval salamanders ranged from 0 to 3 years old. New larval recruitment into the population occurred in November and mid March. Two- or 3-year-old larvae may metamorphose in September.

The SVL of larval clawed salamanders greatly varied within the field population. The largest SVL was 44 mm and the smallest was 17 mm. Considering that the growth of clawed salamander larvae in field populations is about 10～15 mm per year (Hayase and Oseki 1983), the age of the larval salamanders in the study population could consist of at least two different groups. Due to the relatively small sample size on each survey day, obvious differences in size distribution of the SVL according to different ages were not clear. Nevertheless, two important aspects of the dynamics of larval clawed salamanders were found: 1) new larval recruitment into the population might occur in both November and mid March, and 2) clawed salamander larvae might metamorphose into adult salamanders in September.

New larval recruitment primarily occurred in November and mid March, making it difficult to determine the breeding season of *O. fischeri*. The recruitment peak in March might not be caused by the

Table 1. Physical parameters of clawed salamander larvae based on their ages

Parameters \ Age	0 year-old (n = 4)	1 year-old (n = 15, 20 for svl)	2 years-old (n = 37, 35 for svl)	3 years-old (n = 12)	Kruskal Wallis test, χ^2, *df* = 3
Total length (mm)	37.94 ± 0.49 (36.65～39.05)	45.31 ± 2.20 (37.80～66.00)	55.77 ± 1.53 (39.60～69.30)	64.30 ± 2.21 (48.20～72.80)	32.31, $P < 0.001$
Snout-vent length (mm)	21.55 ± 0.46 (20.75～22.80)	24.65 ± 0.87 (20.20～34.10)	30.08 ± 0.77 (20.00～37.30)	34.62 ± 1.11 (27.00～39.50)	29.38, $P < 0.001$
Head length (mm)	6.43 ± 0.32 (5.55～7.05)	7.45 ± 0.40 (6.00～11.20)	9.06 ± 0.24 (6.35～11.25)	10.38 ± 0.36 (7.70～11.60)	26.62, $P < 0.001$
Head width (mm)	4.39 ± 0.07 (4.30～4.60)	5.05 ± 0.20 (4.20～6.80)	6.08 ± 0.16 (4.20～7.40)	6.59 ± 0.23 (5.00～7.65)	24.29, $P < 0.001$
Tail width (mm)	4.24 ± 0.20 (3.70～4.65)	5.01 ± 0.20 (4.00～6.95)	6.02 ± 0.16 (4.40～7.90)	6.70 ± 0.23 (5.10～7.70)	26.40, $P < 0.001$
Body weight (g)	0.28 ± 0.03 (0.2～0.3)	0.55 ± 0.09 (0.3～1.4)	0.98 ± 0.07 (0.2～1.7)	1.38 ± 0.13 (0.5～1.9)	26.36, $P < 0.001$

simple emergence of many larvae from remote areas where they hatched in November into open stream areas. In this study, we found individuals in the population who were 0 years old in October, indicating that they would have hatched in spring, following late autumn breeding. There are two possible explanations for the two peaks in larval recruitment. The first is that *O. fischeri* might breed twice a year in June and late October, retrospectively considering that about 150 days are necessary for eggs to hatch (Iwasawa and Kera 1980, Akita 1989, 1991). In previous studies, Akita (1989, 1991) and Akita and Miyazaki (1991) proposed that Japanese clawed salamanders breed twice a year in both May and October per every two or three years, which they determined based on the presence of sexually developed adults in the population. New larval recruitment of the Japanese clawed salamander in the Tsukuba Mountains occurred through April and May (Hayase and Yamane 1982), showing that adult Japanese clawed salamanders might breed in late autumn (Akita 1983). The second possibility is that *O. fischeri* might breed once a year in June or July, and eggs oviposited and fertilized in that time pass through the winter and hatch in the spring. Previous reports on the breeding of Japanese clawed salamanders (Thorn 1968) and long-tailed clawed salamanders (Serbinova and Solkin 1995) proposed a single breeding season per year in June and July and were consistent with a recent field observation of clawed salamanders breeding in Samcheok, South Korea (Park 2005). We still do not know how long fertilized eggs persist without hatching in the field or how long a breeding season extends in different populations. To determine whether *O. fischeri* breeds once or twice a year, confirmation of the presence of eggs during the winter period and determination of the ages of larval clawed salamanders in both November and March are necessary.

The age of clawed salamander larvae caught in October ranged 0 to 3 years old. Finding 0-year-old individuals indicated that some larval individuals hatched in the previous spring, although that number was as small as 4. This small number of 0-year-old larvae suggests that the size of the possible breeding population in autumn is not large if it exists. Comparing physical parameters revealed that the parameters between 0- and 1-year-olds and between 2- and 3-year-olds were not different. At the moment, there are two possible explanations for this. First, if *O. fischeri* breeds twice a year, larval individuals hatched in November and March have only a 4-month difference in their actual ages although their year ages are one-year different. Second, there is a possibility that the growth rate of clawed salamander larvae during the late larval period may exceed that during the early period. Although the relationship between age and SVL is strong in the field population, the size of larval salamanders cannot be used as an indicator of a specific age because there was great size overlap between individuals of different ages. Due to the complex pattern in breeding season of this species in different areas and populations (Hayase and Yamane 1982, Kozik and Truberg 1985, Akita 1991), several skeletochronological studies of larval salamanders caught at several different times of the year are necessary to determine the exact age structure of a larval population. Although previous studies suggested that the skeletochronological method must be carefully applied to determine the age of clawed salamander larvae due to their possible lack of winter hibernation (Castanet and Smirina 1990), in this study we could reliably determine the age of larval salamanders.

Larval clawed salamanders may metamorphose at 2 or 3 years old in September. Our field survey showed that the number of larvae with an SVL larger than 28 mm was greatly decreased before early October compared to the number in early September. This reduced number of such large individuals was maintained through May and June. This result could be caused by large larval salamanders metamorphosing into adult salamanders. Previous studies suggested that Japanese clawed salamander larvae metamorphosed at 3 years old in June (Hayase and Yamane 1982). Long-tailed clawed salamander larvae metamorphosed at 2 or 3 years old between July and August in Russia depending on the areas and populations studied (Emelianov 1940, 1947, Regel and Epstein 1975, Won 1971, Kozik and Truberg 1985, Smirina et al. 1994). Considering these previous and current results, clawed salamander larvae might metamorphose when they are at least more than 2 years old in late summer or early autumn. When analyzing the field survey results, one should keep in mind that several individuals with an SVL greater than 38 mm were found in the population over the entire study period. At the moment, we do not know why these large individuals had not metamorphosed. They might simply be large one- or two-year-old individuals and could wait another year before metamorphosing at 3 years old. To determine exactly when clawed salamander larvae metamorphose, we need to conduct a longitudinal study of larval individuals for whom we know the time of birth.

In conclusion, our study suggests that clawed salamander larvae metamorphose in September at 2 or 3 years old. Whether or not *O. fischeri* breeds twice a year is not clear in this study, although we detected two main larval recruitment peaks in November and mid-March. In order to determine the exact larval period of this species, we need to determine the exact breeding season in many different populations, and we need to conduct skeletochronological studies to determine the age structure of clawed salamander larvae at several different times of year.

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