

## The Different Growth Rate by Predation Risk in Larval Salamander, *Hynobius leechii*<sup>1</sup>

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### 한국산 도롱뇽의 포식압 유, 무에 따른 성장률의 변화<sup>1</sup>

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#### ABSTRACT

This study examined the different growth rate of larval salamander *Hynobius leechii* by the predation risk. We collected salamander's eggs from Mountain Inwang. Eggs were treated by two different conditions: (1) cue - which had a predation risk three times a day; (2) no cue - which had no predation risk. Predation risk was conducted by using chemical cue from Chinese minnows. The chemical cue treatment started from the next day after the collection and ended one week after the hatching. Between the first week and the second week after the hatching we fed salamander larvae with tubifex ad. libitum. After the treatment phase, we measured snout - vent length of the each larva on the first week and the second week after the hatching. The results showed that there was a significant difference in the growth rate by the predation risk. From these results we found that predation risk can cause the different growth rate to the larval salamander and these changes could be affect their mortality.

**KEY WORDS: CHEMICAL CUE, LARVAL SALAMANDER, MORTALITY, CANNIBALISM**

#### 요 약

본 연구는 포식압에 따라 서로 다르게 나타나는 성장률에 대하여 한국산 도롱뇽 유생을 대상으로 이루어졌다. 인왕산에서 채집해온 도롱뇽의 알(난괴)을 포식압 유, 무에 따라 두 그룹으로 나누었다. 포식자 cue로는 버들치의 체취를 이용하였고, 포식압이 존재하는 그룹은 하루 세 번 포식자의 cue에 노출되도록 하였다. 포식자 cue 처리는 알 채집일 다음 날부터 부화 후 1주일까지 하였고, 이후 각 유생의 snout-vent length를 버니어 캘리퍼를 이용하여 측정하였다. 부화 후 1주일부터 2주일 사이의 기간 동안 각 그룹의 유생에게 충분한 양의 실지렁이를 먹이로 제공하였고, 남은 먹이는 제거하였다. 부화 후 2주일이 되는 날 각 유생의 snout-vent length를 다시 한 번 측정 한 후, 각 개체의 성장률을 계산하여 데이터 분석을 하였다. 그 결과 포식자 cue 처리를 한 그룹은 그렇지 않은 그룹보다 낮은 성장률을 보였으며, 사망률 또한 높은 것으로 나타났다.

주요어: 포식자 큐, 카니발리즘, 한국산 도롱뇽 유생, 생존률

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## INTRODUCTION

In amphibians, both size at and time of metamorphosis have been found to affect later fitness through effects on juvenile survival and adult fecundity (Smith, 1987; Berven, 1990; Scott, 1994; Altwegg and Reyer, 2003). And growth rate of larval amphibians are affected largely by the predator. Predators lower prey density by killing members of the prey population, which can improve the growth rate of survivors by reducing competition (Ullyet, 1950; Slobodkin, 1962; Morin, 1983; Wilbur, 1988). At the same time, predators decrease the foraging activity or increase refuge use of surviving prey, which has the effect of reducing their growth (Sih, 1987; Lima and Dill, 1990; Kotler *et al.*, 1991; Diehl and Eklov, 1995). According to thinning and activity suppression which described above, there are two mechanisms which potentially act in opposite directions on the growth rate of individuals with in a single prey population (Buskirk and Yurewicz, 1998).

However, changes in predation risk or food availability affects not only the birth and death rates of a focal species, but also the behavioral, morphological, life-historical, or physiological traits of that species (Sih, 1987; Lima and Dill, 1990; Lima, 1998a; 1998b). Because the strength of an interaction between food and predators is likely dependent on the phenotypic traits of interaction species, shifts in trait values, due to variation in food, predation risk, or some other environmental factor, can translate into changes in species abundances (Turner *et al.*, 2000). Prey in nature probably experiences a broad range of temporal patterns of risk. If predators are abundant and usually nearby, then prey might face sustained periods of risk with only occasional periods of safety. In contrast, if predators are sparse and side ranging, then prey might experience low risk with only occasional pulses of danger when predators appear (Sih and McCarthy, 2002). The level of perceived predation risk under natural conditions can vary over time due to seasonal changes in local predator and/or prey guild membership, prey movements through heterogeneous microhabitats, and/or movement of potential predators (Sih *et al.*, 2000; Brown *et al.*, 2006). And amphibian embryos detect and respond to olfactory cue. *Ambystoma taxanum* and *Ambystoma barbouri*'s embryos can detect predator's cue and responded by delaying hatching (Sih and Moore, 1993). Amphibian

larvae are good model organism for studying the relationship between individual traits and ecological performance (Buskirk and Schmidt, 2000).

For these reasons, we conducted an experiment how predation risk has effects on the growth rate of larval salamander, *Hynobius leechii* according to the predation levels in laboratory. We used Chinese minnows as a predator indirectly by extracting their odor. And we made two groups according to exposure levels of chemical cue exposure levels. We exposed predator odor thrice a day from the day of collection to the first week after the hatching and measured their snout-vent length(SVL) to compare.

## MATERIALS AND METHODS

### 1. Study animal and site

Seven of the Korean salamander (*H. leechii*) egg sacs were collected from stream in Mountain Inwang (37°35'58.8" N, 126°58'03" E) between March 4 and April 3, 2010. In general, Korean salamanders lay their eggs at the small pond which generated by the water flowed from the upper side. But in case of Mountain Inwang, thousands of eggs were laid at about 1km long stream located in the small valley with trees. In particular, the salamander eggs were placed in the part of stream where the water flowed slower than other parts of stream or at the center of the stream which has relatively low fluid speed with fallen leaves. After the collection, each egg sacs were kept in plastic container (29cm long×16cm wide×19cm high) separately with the supply of air filled with 3l of dechlorinated tap water. The average room temperature was 16.4°C and the water temperature in the container ranged between 11~20°C through out the experiment. Photoperiod was not controlled during the experiment procedure. The test was started the next day of the egg collection. Most eggs hatched approximately 2 weeks after since the eggs were collected. After the hatching, we made a group of 8~11 individuals and kept each group in the test container to prevent the cannibalism and inducing cannibal morph by high density.

We used Chinese minnows(*Rhynchocypris oxycephalus*) as a predator of *H. leechii*. *R. oxycephalus* is a popular

fish species of Korea distributed in rivers flows to Yellow Sea basin. There are no published research about Chinese minnows ate salamander larvae as a food source. However, they are carnivorous fresh water fish which feeds on fry, insects and other animal food. And they have a similar habitat condition with larval salamander, thus we assumed *H. leechii* larvae could recognize them as a predator and conducted our test.

## 2. Method

### 1) Test procedure

We made two groups of eggs for the different predation risk level; predation risk(cue) and no predation risk(no cue). Predation risk group received thrice a day chemical cue treatment at 1000, 1300, and 1600h and no predation risk had any cue that they could realize as a predation risk. Each treatment include 2ml of chemical cue following the well-established protocol (Brown *et al.*, 2007). When we treated the test animal, we injected the chemical cue along the container's wall not to disturb the test animals by the water waves. Chinese minnows were used as a predator indirectly by extracting their odor. We captured three Chinese minnows from a pond in Mountain Bulam and fed them with commercial fish flake every other day. Predator cue was made everyday with 500ml dechlorinated tap water and one Chinese minnow by containing it for overnight so that fish odor could percolate to the tap water (Ferrari and Chivers, 2008; 2009). Chemical cue source animal was changed everyday not to get the animals stressed. We conducted our test explained above from the next day of collection to the first on week after the hatching. We started to feed larvae when each larva reached to 7day old ad. libitum during the second week after hatching. We measured each salamander larvae's snout-vent length (SVL) on the 7th and 14th day after the hatching. When we analyze the data from measuring, we calculated SVL growth rate of each larvae by subtracting the first SVL from the second one and divided it by the second SVL. Every growth rate was converted into percentage. When we started our test, we had 152 individuals of larval salamander. However, some of test animal died between the first week and second week after hatching because of high pressure of predation risk and

Table 1. The number of larvae and test group used for final statistical analyze

	Total number of larvae	Test group numbers
Cue	41	4
No cue	47	5

starvation. Thus, we excluded individuals which died during the test treatment and used remained individuals when we analyze our data(Table 1.). Also, we calculated each mortality rate of cue and no cue groups into percentage to analyze.

### 2) Behavioral test

We conducted behavioral test apart from the main treatment to find out behavioral differences between chemical cue treated group and no cue group. First we made 8 and 10 groups of salamander larvae with 5 individuals each which had any experience about predators after the collection in order to test the activity rate changes by the predation risk e (Table 2). And we contained groups of 5 individuals in 500ml beaker filled with 250ml dechlorinated tap water and left for 10 minute to acclimate. In the middle of the beaker, we draw a line along the beaker diameter to measure the number of activity rate by counting each larva's number of line crosses during the test. After the conditioning phase, we recorded 20min before and after 10ml of the cue or tap water injection. Cue was the same predator's cue that used during the treatment phase, and tap water was the same one which filled the test beaker. When we analyze the data, we counted the number of line crosses by larval salamander in the beaker. All the test procedure was followed well established test method by M. C. O. Ferrari (2008).

### 3) Statistical analysis

To analyze data from the experiments that nine groups of larvae, we used independent t - test to determine significant changes in growth rate between groups. And we also used independent t-test to find out significant

Table 2. Details of behavioral test group.

	Number of larva	Number of group
Water	40	8
Predator's cue	50	10

differences in activity rate between chemical cue treated and control group. All tests were significant in alpha range 0.05 and all statistical analysis were performed using SPSS 18.0 (SPSS, Chicago,IL,U.S.A)

## RESULTS AND DISCUSSION

### 1. Results

#### 1) Mortality rate according to treatment group

The result showed that the group of predation risk treated had higher mortality rate than no predation risk treated group between first week and second week after hatching (Table 3).

#### 2) Mean snout-vent length between first week and second week after the hatching

The results of descriptive statistics analysis on snout-vent length between the very first week and second week after the hatching were described below with figure (Figure 1, total number of survived larvae in the second week = 88). And actual mean of SVL on the first week of chemical cue treated group were higher than no cue

group ( $p < 0.05$ ,  $t = 8.558$ ). However, there was no significance in mean SVL at the second week between cue group and no cue group after hatching.

#### 3) Changes in growth rate by the predation risk

Larval salamander showed significant growth rate changes by the predation risk level between first week and second week after hatching ( $n = 88$ ,  $t = 5.263$ ,  $< 0.05$ ). Especially, there was significant difference in growth rate between cue group and no cue group (Figure 2). The mean growth rate of cue group was  $15.22 \pm 8.24$  and no cue group was  $27.35 \pm 12.59$ .

#### 4) Decreased activity by the predation risk

We compared the mean activity rate of no cue and cue group. The results of independent t - test showed no significance before the cue or water injection, however, it came out there was a significant difference between chemical cue treated group and control group (Figure 3.  $p < 0.01$ ,  $t = 5.598$ ). Mean number or line cross of cue treated group was  $21.2 \pm 15.89$  and no cue group was  $148.88 \pm 70.43$ .

### 2. Discussion

#### 1) Different growth rate and activity rate by the predation risk

Organisms that use ephemeral habitats often show such a "fast" lifestyle; including shorter larval periods than

Table 3. Mortality rate according to predation risk

	Mortality rate(%)
Cue	53.41
No cue	26.56

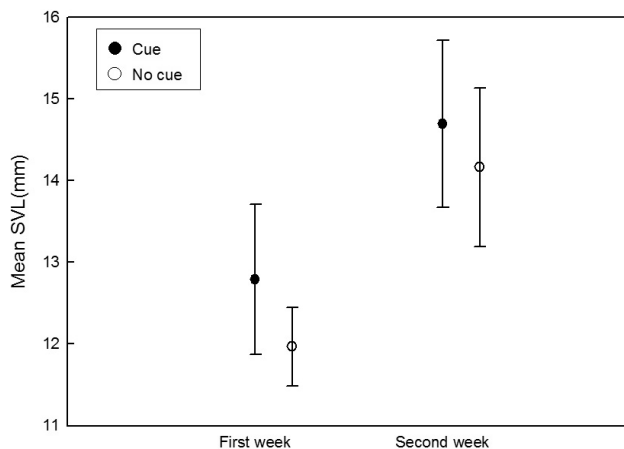


Figure 1. Mean SVL of the first week and the second week

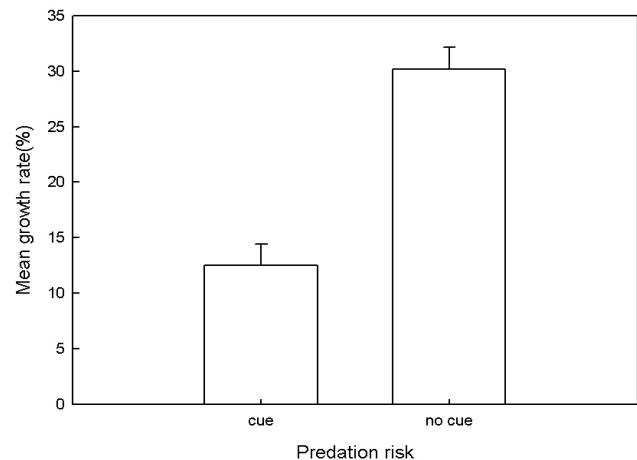


Figure 2. Mean growth rate by the predation risk levels with standard error bar

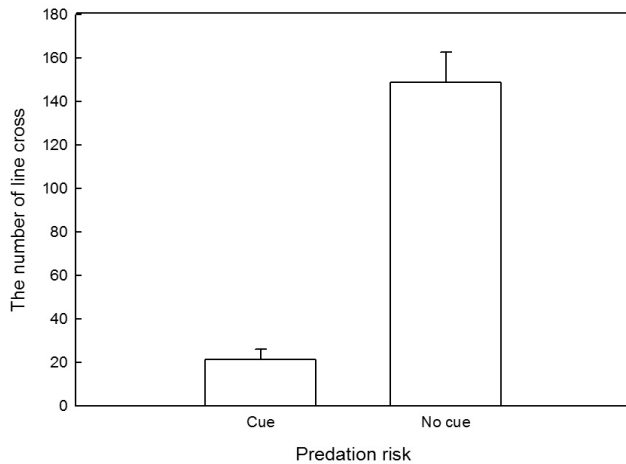


Figure 3. Average number of line crosses of no cue and cue groups with standard error bar

those in more permanent habitats (Wilbur, 1980; 1984; Sih, 1987). In general, larval amphibians of Korean including *H. leechii*, breed in ephemeral habitats where the circumstances are far scarce than permanent stream. Therefore they have to show great growth rates to reach faster metamorphosis for breeding success. From our results, we found significant changes in growth rate by the predation risk. Especially, predation risk which had thrice a day predation risk treated group showed fairly weak growth rate than no predation risk treated group. The presence of predators often reduced both survival and growth of their prey and this was caused by larvae's reduced feeding rates (Mcpeck, 1990a; 1990b; 1997; 1998; Pechkarsky *et al.*, 1993). And responding to chemical alarm cues of both conspecifics and sympatric heterospecifics by reducing the time spent engaged in other fitness related behaviors such as foraging or mating significantly increases individual survival (Chivers *et al.*, 1996; Mathis and Smith, 1993; Mirza and Chivers, 2000; 2003) but such responses are costly. Our perspective about low activity at the conditions of predator's cue is similar to the hypothesis described above. Thus we suppose that under the high predation circumstance, larval salamander might not have enough time to foraging activity by reducing it not to attract the predator's attention. And the result of activity rate test, which showed reduced activity at the predator's chemical cue treated group, support this assumption.

## 2) Different growth rate, SVL and mortality by predation risk

In some cases, foraging rate can increase with size faster than metabolic costs. This may be due to increased performance of the feeding apparatus (Peters, 1983), or over coming gape limitation and utilizing new food resources unavailable to smaller individuals (Nakamura and Kasahara, 1956; Smith and Petranka, 1987; Osenber *et al.*, 1992). Larger individuals may also actively suppress the feeding activity of smaller individuals (Crowley *et al.*, 1987; Smith 1990). For example, large plants can shade smaller competitors (Weiner, 1990), and large animals can defend territories with higher resources (Baird *et al.*, 1996; Iguchi, 1996), or steal food (Rivault and Cloarec, 1990). However, the mean snout-vent length of the chemical cue treated group was significantly higher than the other at the first week after the hatching. And we could see there was very slight differences between two groups at the second week. From these results, we can suppose that larvae in the predation risk circumstance had larger SVL than the other groups at no predation risk circumstance so that taking a advantage to forage and avoiding predator, but grew slowly due to heavy predation risk while the other grew faster. However, enlarging their body in the early hatchling without food resource is too cost to the larval salamander and could be fatal. During the chemical cue treatment phase, we didn't supply any food to the larval salamander and we had the result that predator cue received group showed higher mortality than the other group. Thus we could confirm this prior premise by showing that high mortality at the predator cue treated group. In other words, they didn't have any food supply during the first week treatment.

In amphibian, the optimal theory avoiding aquatic predator is migrate to the new habitat through metamorphosis. And there might be a minimal optimal size which determined by growth/predation trade-off causing high survival rate to the terrestrial life. In our case, we could infer that larvae in the predation risk hatch in larger size than the other to be more developmentally advanced hatchlings to escape well from the initial attack (Petranka *et al.*, 1987). But under the high predation pressure, they could not forage actively, thus they could not help showing low growth rate than the larvae in the no predation risk.

Our study had a limitation that we did not study until the salamander larvae's metamorphosis and compare to general growth rate in nature. However, we suppose that larvae in the predation risk might show early metamorphosis than the other group besides their slow growth rate, and we suggest further study should be needed about this point.

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