

Cannibalism in the Korean Salamander (*Hynobius leechii*: Hynobiidae, Caudata, Amphibia) Larvae

Shi-Ryong Park, Ji-Young Jeong and Daesik Park^{1*}

Department of Biology Education, Korea National University of Education, Chungbuk 363-791, Korea; ¹Department of Science Education, Kangwon National University, Kangwon 200-701, Korea

Abstract: Cannibalism plays important roles at the levels of both individual and population. To enhance overall rate of successful survival and reproduction, salamander larvae may have evolved to consume both conspecifics and heterospecifics. Consuming conspecifics could result in decreased inclusive fitness possibly by killing relatives. In several salamander species, discrimination of salamander larval siblings from non-siblings and heterospecifics to avoid such a risk has been reported. To determine whether the Korean salamander larvae consume non-siblings more often than siblings and to analyze prey preferences of the salamander larvae in several different experimental conditions, a series of foraging experiments was conducted in the laboratory. We found that 1) large cannibal larvae preyed on small sibling more often than small non-sibling in a mixed group of sibling and non-sibling, 2) cannibal larvae preferred to consume live, weak, and small larvae to dead, healthy, and large larvae, and 3) cannibal larvae consumed heterospecific tadpoles more often than conspecific non-sibling larvae in a mixed group. In addition, the larval density was positively correlated with the occurrence of spacing behavior, one of the agonistic predator behaviors among salamander larvae.

Key words: *Hynobius leechii*, cannibalism, kin recognition, salamander larvae, spacing behavior

Cannibalism functions at the levels of both individual and population. In amphibians, larval-larval cannibalism is widely spread (Blaustein and O'Hara, 1982; Pfennig and Collins, 1993; Zhang et al., 2003; Gibbons et al., 2003). Cannibals show faster growth, larger size, higher survivorship or enhanced reproduction compared with the larvae that do not show cannibalism (Fox, 1975; Polis, 1981). Since

cannibalism often acts as an intraspecific predation in a population, it plays important roles in the regulation of population structure and dynamics through the control of disease spread and the alteration of population size and age structure (Fox, 1975; Polis, 1981; Ziemba and Collins, 1999).

Several potential costs of cannibalism have been suggested. First, cannibalism of sibling or close relatives could reduce inclusive fitness, defined as the sum of direct reproductive success of an individual (the number of offspring that the individual produces, so that the offspring contains the half of the individual's gene) plus indirect reproductive success of the individual (the number of offspring who has the gene shared with the individual's gene based on genetic relatedness) by increasing successful reproduction of relatives (Hamilton, 1964). Thus, to avoid sibling or close relative cannibalism, salamander larval cannibals discriminate close relatives from non-related larvae and conspecifics from heterospecifics (Waldman, 1991; Walls and Roudebush, 1991; Pfennig et al., 1993). Second, because of genetic similarities among conspecifics, cannibalism may have a high chance to spread harmful diseases among conspecifics (Pfennig et al., 1998). Third, possible injury of both cannibal and prey larvae by cannibalism can occur, subsequently resulting in the increased rate of death (Elgar and Crespi, 1992). Thus, the occurrence of cannibalism can be affected by several different factors such as food availability (Walls, 1998), individual density within an area (Nishihara, 1996), size variations within a population (Michimae and Wakahara, 2002), and the presence of heterospecifics (Maret and Collins, 1996). Recent studies have proposed that overall occurrence of cannibalism may be context-dependent at a given time (Reeve, 1989; Waldman, 1991; Hokit et al. 1996). Despite these previous studies, detailed factors involved in cannibalism are not fully understood.

Prey propensity of the Korean salamander, *Hynobius*

*To whom correspondence should be addressed.
Tel: 82-33-250-6739, Fax: 82-33-242-9598
E-mail: parkda@kangwon.ac.kr

leechii, has been studied in adults (Yoon et al., 1996). They prefer live preys and generally eat earthworm, insects, and aquatic invertebrates. While studies on cannibalism in salamanders could increase our understanding of population dynamics, little is known about the prey preferences and foraging behaviors of *H. leechii*'s larvae. It was only reported that the Korean salamander larvae often consume conspecifics (Yoon et al., 1996).

The purpose of this study was to determine whether the Korean salamander larvae consume non-siblings more often than siblings and to analyze prey preferences between live and dead preys, weak and healthy preys, small and large preys, and conspecifics and heterospecifics. In addition, we studied if the larval density is correlated with the occurrence of spacing behavior, one of the agonistic predator behaviors among salamander larvae, defined as the behavior of sudden body twist of a larva with biting displays as other larvae approach towards the larva.

MATERIALS AND METHODS

Animal collection and rearing

Thirty nine pairs of the Korean salamander (*H. leechii*) egg sacs and more than five clutches of frog (*Rana dybowskii*) eggs were collected from rice fields and small streams in Gaduk, Chungbuk (36°33' N, 127°35' E), and Ansong, Cheonbuk (34°49' N, 127°42' E), between March 21 and May 30, 2004. The egg sacs and eggs were separately kept in glass aquaria (40 long × 25 wide × 16 cm high) with the supply of air. The water temperature in the aquaria ranged between 14–16°C throughout the experiments, and the photoperiod was not modified. Most eggs hatched approximately 2 weeks after their collection. The hatching rate of the salamander eggs was 94.46% in this study and was similar to the previous report (Park et al., 1996). Approximately, a quarter of offspring was arbitrarily classified as small prey larvae and was kept separately from the others using a mesh screen box (15 long × 12 wide × 5 cm high) placed in the aquaria.

To prevent sibling cannibalism, we supplied plenty of *Limnodrilus gotoi* into the aquaria every day. To keep the size of prey larvae as small in the mesh screen boxes, we supplied only quarter amounts of the food that we provided to the cannibal (predator) larvae. The water in the aquaria was changed every 3 or 4 days, and at the time each aquarium was completely washed with tap water.

Sibling discrimination and prey preference

For experiments, each cannibal (predator) larva was placed in an individual glass jar (8 cm in radius and 10 cm in height) for 20 min for acclimation before each trial began. One hour before this process, we numbered all individuals used in the experiments by the toe clipping or based on

their unique skin patterns. Also, the SVL (snout-vent length) of each larva was measured to the nearest 0.1 mm using a vernier caliper. Each trial was conducted for 12 h from 1000. At the end of each hour during a trial, we observed the amount and type of prey being eaten.

To determine whether the cannibal larvae discriminate siblings from non-siblings as a prey, we set up three different choice experiments; 2 siblings, 2 non-siblings, or one sibling and one non-sibling were exposed to the cannibals. For each experiment, 36 cannibal larvae were allowed to consume one of the two prey items for 12 h. Some of larvae were used again in different experiments after 48 h rest. We didn't notice any differences in the food consumption rate between the first trial and the second trial conducted after 48 h rest (Park et al., unpublished data). If cannibals significantly change prey preference from siblings to non-siblings between the single group of 2 non-siblings or 2 siblings and the mixed group of one non-sibling and one sibling, we considered the result as cannibal larvae discriminate siblings from non-siblings and determined which type of preys was preferred. The average size of the preys was smaller as 19.65 mm SVL than that (SVL = 22.84 ± 2.49 mm, $n = 36$) of the cannibal larvae.

We studied the prey preference of cannibal larvae between live (SVL = 17.54 ± 0.49 mm, $n = 35$) and dead (SVL = 17.19 ± 0.52 mm, $n = 35$), healthy (SVL = 17.29 ± 0.25 mm, $n = 68$) and weak (SVL = 17.33 ± 0.40 mm, $n = 68$), and small (SVL = 15.80 ± 0.24 mm, $n = 32$) and large (SVL = 22.27 ± 0.51 mm, $n = 32$) larvae. Each experiment was conducted for 12 h from 1000 using the same method described above until the cannibal consumed one of the two prey items. Some of larvae were used again in different experiments after 48 h rest. Prey larvae used in this experiment were non-siblings to the experimental cannibals. Weak larvae were arbitrarily selected based on swimming speed and maintaining body balance. Unlike healthy larvae, weak larvae swam slowly, generally could not balance their body during swimming, and often had air bubble in the abdominal cavity (Park et al., unpublished data). The large prey larvae used in this experiment were smaller than the cannibal predator larvae (SVL = 30.41 ± 1.32 mm, $n = 32$).

We studied the prey preference of salamander larval cannibals between conspecific salamander larvae and heterospecific frog tadpoles of *Rana dybowskii*. The cannibal larvae (SVL = 29.48 ± 1.79 mm, $n = 88$) were presented with a choice of two prey items in three different experimental conditions. In two single group conditions, two non-sibling larvae or two tadpoles were presented as prey items. In the mixed group condition, one non-sibling larva and one tadpole were presented. Each experiment was conducted for 12 h from 1000 using the same method described above until the cannibal preyed on one of the two prey items. Cannibal

larvae were used again in different experiments after 48 h rest. If cannibals significantly change prey preference from conspecific salamander larvae to heterospecific tadpoles between the single group and the mixed group, we considered the result as cannibals discriminate conspecifics from heterospecifics, and determined the type of preys preferred. The size of prey salamander larvae and tadpoles used in this experiment were similar (Larval SVL = 16.45 ± 0.19 mm, $n = 88$; tadpole SVL = 16.50 ± 0.17 mm, $n = 88$). In addition, we determined the prey preference of cannibal larvae ($n = 42$) between dead salamander larvae (SVL = 16.27 ± 0.21 mm, $n = 42$) and dead tadpole (SVL = 16.34 ± 0.35 mm, $n = 42$) using the same method.

Spacing behavior

The spacing behavior, one of the agonistic predator behaviors among larvae, is defined as the behavior of sudden body twist of a larva with biting displays as other larvae approach towards the larva. We tested whether or not the larval density is correlated with the occurrence of the spacing behavior. To compose different individual densities, we set up four circular glass jars (8 cm in radius and 10 cm in height) containing 5 (SVL = 17.11 ± 0.28 mm, $n = 5$), 10 (SVL = 17.17 ± 0.40 mm, $n = 10$), 20 (SVL = 17.14 ± 0.34 mm, $n = 20$), and 40 (SVL = 17.16 ± 0.32 mm, $n = 40$) larvae. We measured the number of spacing behavior every 5 min for 140 min and calculated the average frequency of spacing behavior at each given density. Throughout the experiment, we maintained constant larval density in each experimental jar by adding new larvae if cannibalism occurred. Larvae used in this experiment were two or three days old after hatching.

Statistical analysis

To analyze data from the experiments that two groups were compared such as prey preference between live and dead, healthy and weak, and small and large larvae, we used the Chi-square (χ^2) test. To determine significant changes in prey preference from siblings to non-siblings and from conspecifics to heterospecifics between the single group and the mixed group, McNemar test was used (Sokal and Rohlf, 1981). In the test, we reduced alpha to 0.025 according to Bonferroni's inequality because each data set was used twice (Snedecor and Cochran, 1980). Relationship between the larval density and the frequency of spacing behavior was analyzed by the Pearson's correlation (r) test.

RESULTS

Sibling discrimination and prey preference

The frequency of cannibalism was similar between sibling and non-sibling single groups (Fig. 1, $\chi^2 = 0.55$, $P = 0.46$). Cannibal larvae preyed on fewer non-siblings in the mixed

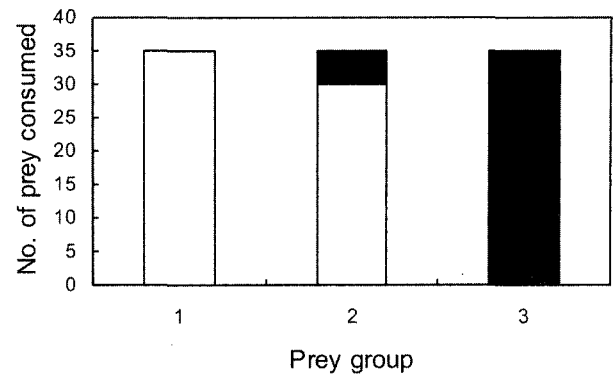


Fig. 1. Larval *Hynobius leechii* preyed on siblings (□) more often than non-siblings (■) in the mixed group (2, $P < 0.01$), but the total number of siblings (□) and non-siblings (■) consumed in the single groups (1, 3) was not different ($P = 0.46$).

group of siblings and non-siblings than in the single group composed of two non-siblings alone (Fig. 1, $\chi^2 = 6.93$, $P < 0.01$).

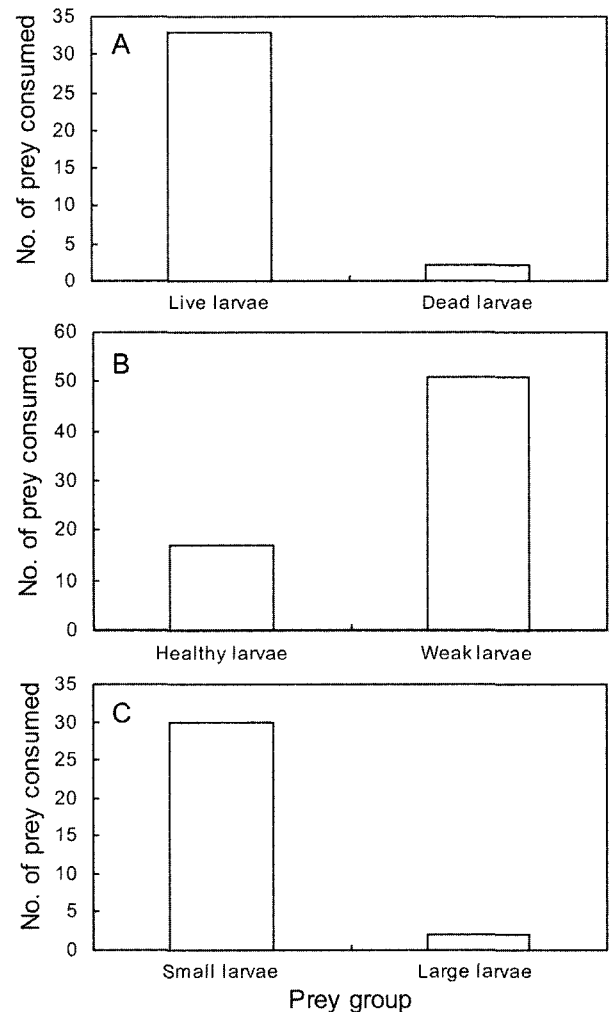


Fig. 2. Larval *Hynobius leechii* preferred to consume live (A), weak (B), and small (C) conspecifics to dead, healthy, and large conspecifics (for all cases, $P < 0.01$).

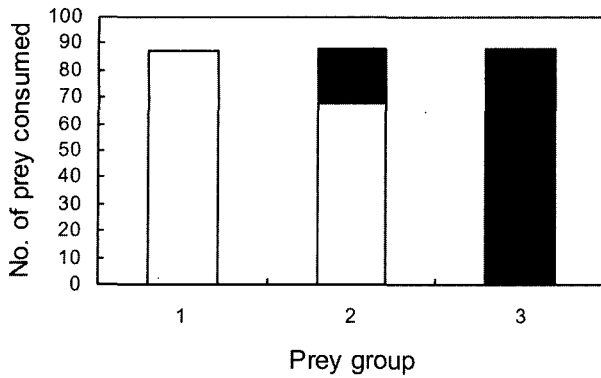


Fig. 3. Larval *Hynobius leechii* preyed on heterospecific tadpoles (□) more often than conspecific non-sibling larvae (■) in the mixed group (2, $P < 0.01$), but the total number of tadpoles (□) and salamander larvae (■) consumed in the single groups (1, 3) was not different ($P = 0.11$).

Cannibal larvae preferred to consume live to dead (Fig. 2A, $\chi^2 = 27.46$, $P < 0.01$), weak to healthy (Fig. 2B, $\chi^2 = 17$, $P < 0.01$), and small to large (Fig. 2C, $\chi^2 = 24.5$, $P < 0.01$) larvae.

Cannibal larvae preyed on similar amounts of conspecific salamander larvae or heterospecific tadpoles ($n = 87$ larvae, $n = 88$ tadpoles; Fig. 3, $\chi^2 = 2.56$, $P = 0.11$) in each single group of salamander larvae and frog tadpoles. However, cannibal larvae preyed on fewer conspecific salamander larvae in the combined group of conspecific salamander larva and heterospecific tadpole, than in the single group composed of salamander larvae alone (Fig. 3, $\chi^2 = 27.73$, $P < 0.01$). However, the different preference was removed when both dead larvae and tadpoles were given as preys (data not shown, $\chi^2 = 0.10$, $P = 0.76$).

Spacing behavior

The frequency of spacing behavior was significantly correlated with larval density within the experimental jars (Fig. 4, $r = 0.94$, $P < 0.01$).

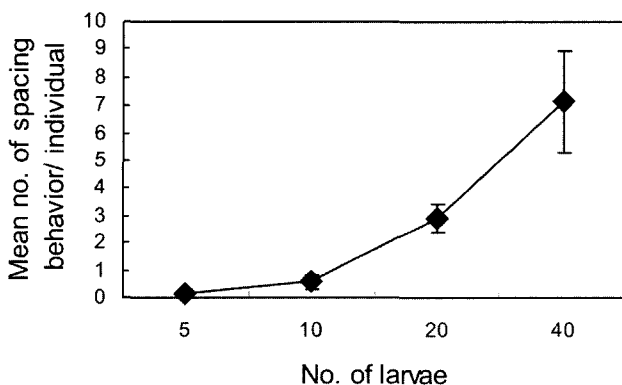


Fig. 4. The occurrence of spacing behavior, defined as the behavior of sudden body twist of a larva as other larvae approach towards the larva, was positively correlated with the individual density ($r = 0.94$, $F < 0.01$). Mean \pm SE.

DISCUSSION

Our results indicate that larval *H. leechii* discriminate siblings from non-siblings, and small siblings are more vulnerable to cannibalism by large siblings. Sibling cannibalism was also reported in the marbled salamander *Ambystoma opacum* (Walls and Blaustein, 1995). Large larval *A. opacum* consumed small siblings more often than small non-siblings when both siblings and non-siblings were simultaneously presented as prey items. The sibling cannibalism was not due to size, behaviors, or different foraging efficiencies between siblings and non-siblings. The preference of sibling cannibalism may be explained by the combined effects of behavioral aspects of siblings and inclusive fitness of sibling cannibalism. When the marbled salamander larvae were presented to similar-sized siblings and non-siblings, they showed submissive behaviors only towards the siblings, but not towards the non-siblings (Walls and Roudebush, 1991). If smaller siblings also show submissive behaviors to larger siblings, larger siblings who intend to prey on may readily consume siblings without paying potential costs of injury by cannibalism, consequently resulting in increased fitness of the cannibals. In harsh environments such as the temporary ponds where salamander larvae usually inhabit, small salamander larvae often have a low expectancy of survival (Waldman, 1991; Crump, 1992). Considering smaller siblings may have a high chance being eaten by non-siblings or by heterospecifics in such environments, consuming small siblings may less impair the inclusive fitness of the cannibals compared to preying on similar or larger size siblings. Thus, although salamander larvae can discriminate siblings, sibling cannibalism may occur based on different contexts (Waldman, 1991; Crump, 1992; Gibbons et al., 2003). For detailed explanation of how sibling cannibalism satisfies the kinship theory of Hamilton (1964), which proposes that salamander larvae will avoid siblings as a cannibal prey if alternative preys are available, further studies are necessary.

Korean salamander larvae may increase successful cannibalism by selecting small and weak larvae as preys. In our experiments, larval salamanders significantly consumed smaller and weaker non-siblings than larger and healthier non-siblings. The preference of small and weak larvae to large and healthy larvae may be advantageous to cannibal larvae in two ways. First, the small and weak prey may decrease the risk of cannibalism, given that one of the potential costs of cannibalism is a possible injury or death from retaliation by the victim (Elgar and Crespi, 1992). In several salamander species, it is known that risks to preying on small and weak larvae are much lower than large and healthy larvae (Elgar and Crespi, 1992). Second, the preference of small and weak larvae as preys may allow the predator larvae to achieve effective cannibal. In many salamander larvae, the rate of successful predation depended

on the prey size (Brodie and Formanowicz, 1983; Stenhouse et al., 1983) and the number of larvae eaten at a given time was negatively correlated with the size of larval preys (Wakahara, 1997; Zhang et al., 2003). Larval *H. nebulosus* spent much more time to swallow large and healthy larvae than small larvae and the size of cannibals always exceeded the size of the victim (Kusano et al., 1985).

Preference of heterospecific preys to conspecific larvae may be explained by the inclusive fitness theory of Hamilton (1964). In our study, cannibal larvae significantly consumed more tadpoles than salamander larvae in a mixed group although the number of total individuals being eaten in each single group of salamander larvae alone and tadpoles alone was not different. Many species of salamanders including *H. leechii* breed in small or ephemeral ponds (Lannoo and Bachmann, 1984; Park et al., 1996; Wildy et al., 1998; Park and Park, 2000) so that there is a chance for sibling cannibalism, particularly during dry season (Walls, 1998). Cannibal larvae may conserve indirect component of their inclusive fitness by not consuming close relatives (Hamilton, 1964; Waldman 1988), particularly if alternative non-related or heterospecific preys are available. For example, *A. tigrinum* larvae were more likely to consume distant relatives than close kin (Lannoo and Bachmann, 1984). In larval *A. t. nebulosum*, the frequency of cannibal morphs was correlated with the density of chorus frog tadpoles, *Pseudacris triseriata*, but not with the density of the salamander larvae, showing that cannibals prefer to prey on heterospecific tadpoles (Loeb et al., 1994). Preference of non-related conspecifics or heterospecifics as preys in *H. leechii* could increase the inclusive fitness of the cannibals.

In *H. leechii* larvae, spacing behavior may function to avoid potential risks of cannibalism by conspecifics. Although we did not study fighting behaviors of the Korean salamander larvae, we often observed the larvae of which tail was beaten in stock tanks, indicating that larvae can damage each other (Park et al., unpublished data). In many salamander species, cannibal larvae show agonistic behaviors in the form of intraspecific biting and cannibalism to avoid cannibalism (Walls and Roudebush, 1991; Camp and Lee, 1996). Adults *Desmognathus quadramaculatus* bit and ate juveniles, larger juveniles ate smaller juveniles, juveniles fled from adults, and wandering salamanders of similar size were aggressive approximately 50% of the time (Camp and Lee, 1996). Since increased population density commonly results in high rate of cannibalism in larval salamanders (Michimae and Wakahara, 2002; Wildy et al., 2001), to avoid potential harmful risks from conspecifics, cannibal larvae should give more attentions to neighbor larvae as individual density increases. In our experiment, the result that the frequency of spacing behavior was positively correlated with the conspecific individual density suggests that the high frequency of spacing behavior by increased

larval density may somewhat decrease the potential risks of cannibalism among conspecifics.

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