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Faster Growth of Head Size of Pre-feeding Larvae in a Cannibalistic Population of the Salamander *Hynobius retardatus*

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ABSTRACT—Cannibalism is common in high density larval populations of *Hynobius retardatus*. Because cannibals are gape-limited, possessing a wider head (mouth) may be advantageous in these populations. Field observations showed that the pre-feeding stage larvae were more vulnerable to cannibalism than feeding stage larvae in a high-density larval population. The data also showed that larvae with proportionally smaller head widths are more vulnerable to cannibalism than those with larger heads. Therefore, faster growth of head size during pre-feeding stage is predicted to be favored in a population with frequent cannibalism. A laboratory comparison revealed that head growth (proportionate change in head width to body length) during the pre-feeding stage was greater in the larvae of a cannibalistic population than in those of a non-cannibalistic population. These results support the hypothesis that a wide head is an adaptation against frequent cannibalism in larval *H. retardatus*.

INTRODUCTION

Cannibalism between juveniles is common in carnivorous animals (reviewed in Elgar and Crespi, 1992) and is assumed to be caused by competition for food (Fox, 1975; Polis, 1981). Many larval salamanders that compete intensely for food are cannibalistic (reviewed in Crump, 1992). Larger head and mouth width are advantageous to cannibals, because their predation is gape-limited (Kusano *et al*., 1985; Ohdachi, 1994). The disproportionately large head, wide mouth, and elongated vomerine teeth in cannibalistic morphs of *Ambystoma* larvae (Powers, 1907) are induced by high rearing density (Collins and Cheek, 1983), as well as by factors such as kinship (Pfennig and Collins, 1993) and diet (Walls *et al*., 1993). My previous studies showed that large head size among *Hynobius retardatus* larvae is also induced by high larval density (Nishihara, 1996a, b). The relative head widths to body size of feeding stage (stages 48 to 54; Iwasawa and Yamashita, 1991) larvae collected from Erimo, in Hokkaido Prefecture, where the egg density is high and cannibalism occurs, are larger than those of larvae collected from Bankei, in Hokkaido Prefecture, where density is low and cannibalism is not observed (Nishihara, 1996a). In captivity, the heads of feeding stage larvae from both populations grow to be proportionately larger when reared under high density conditions even when physical interactions are prevented (Nishihara, 1996a, b).

Faster growth of head size during pre-feeding stage is predicted to be favored in a larval salamander population with frequent cannibalism. This prediction is based on two hypotheses. First, a larger head in pre-feeding stage could reduce the probability of being caught and swallowed by conspecific cannibals because of the gape limitation. The larvae of *Hynobius nebulosus* have high mortality rates just after hatching due to predation and cannibalism (Kusano, 1981), and this is also predicted to be the case in *H. retardatus*. Second, faster growth of head size during pre-feeding stage could increase the probability of being able to cannibalize other individuals once the larva reaches the feeding stage. Reilly *et al*. (1992) argued that the time when a wider head is most important to a cannibal morph is during the first cannibalistic event among the similar-sized small larvae. These two hypotheses are not mutually exclusive, and if at least one of them is supported, I should predict that the relative growth rate of head size to body size during pre-feeding stage is larger in a population with frequent cannibalism than that with infrequent cannibalism.

In this paper, I first consider the above hypotheses on pre-feeding stage larvae having the advantage of possessing larger heads. I test whether pre-feeding stage larvae are more vulnerable to cannibalism than feeding stage larvae, whether the larvae with relatively smaller heads to body size are more vulnerable to cannibalism than those with relatively larger heads, and whether those with relatively larger heads are more cannibalistic than those with relatively smaller heads. These are tested in natural habitat where cannibalism is frequent. Second, I analyze whether my prediction on larval head growth pattern is correct. I compare the pre-feeding stage growth

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patterns under the laboratory controlled experiments (Nishihara, 1996a, b) between populations with high and low frequency cannibalism. I test whether the effect of population on relative growth rate of head width to snout-vent length is significant.

MATERIALS AND METHODS

Field observations

Larvae feeding on conspecific larvae were collected from a pond in Meguro of Erimo-cho (42°07'N, 143°16'E; Erimo population), where frequent cannibalism was observed (Nishihara, 1996a). Observations were made from one side of the pond between 10:30 AM and 4:30 PM on June 17, 1996. Whenever cannibalism was observed, I picked up the cannibal and its victim immediately. I collected 18 cannibal/victim pairs in total. The victim was carefully pulled out from the mouth of its cannibal. All the victims except two were still alive and were able to swim in the water. Their physical damage was not obvious. For the control, 30 larvae were sampled randomly by dip-netting the pond swiftly. After placing the net on the ground, I collected several individuals each from the net arbitrarily until a total of thirty individuals were obtained. Their snout-vent length (distance from the anterior tip of snout to the posterior margin of vent, to the nearest 0.01 mm) and head width (maximum width across the dorsal side of the head, to the nearest 0.01 mm) were measured alive using a pair of digital vernier calipers. Their developmental stages were recorded based on Iwasawa and Yamashita (1991).

Proportion of the number of larvae at pre-feeding stage to that at feeding stage was compared between victims and randomly collected larvae. Relative head width to snout-vent length was compared between victims and randomly collected individuals, and between cannibals and randomly collected larvae by Analysis of Covariance (ANCOVA). Because the size changes as they develop, the comparisons were made between individuals within the same ranges of developmental stages.

Laboratory experiments

Eggs from nine clutches each were collected within 24 hr of oviposition from the pond in Meguro of Erimo-cho (described above) and a pond located near Horomi Pass of Bankei (43°2'N, 141°18'E; Bankei population) in 1994 and 1995, respectively. Since egg density is higher in Erimo population than in Bankei population (Nishihara, 1996a, b), the experimental individuals were reared under both high- (nine individuals per container of 18 × 18 × 6.5 cm) and low-density (one individual per container of the same size) conditions during the pre-feeding stage. Pre-feeding stage is defined as non-feeding stage after hatching, which corresponds to the stages from 41 to 43 based on Iwasawa and Yamashita (1991). Three and 18 containers were set for the high- and low-density treatments, respectively. Three and two individuals in high- and low-density treatments, respectively, from the Erimo population, and one individual in high-density treatment from the Bankei population died and were excluded from the later analyses. Within the high-density treatment of each population, no differences were found in snout-vent length or head width growth among individuals reared in different containers (Nishihara, 1996a, b). Consequently, individuals subjected to high-density treatment were pooled in the subsequent analyses. The final sample sizes were thus, 24 and 16 for high- and low-density treatments, respectively, for the Erimo population, and 26 and 18 for high- and low-density treatments, respectively, for the Bankei population. Larvae from nine different clutches were assigned to containers randomly, in a way that minimizes sibship effects between treatments and among high density containers (Nishihara, 1996a). Conditions of LD 15:9 photoperiod and a constant temperature of 17°C were maintained throughout the experiment.

Containers were made of transparent acrylic plates (0.5 cm thick), and nonchlorinated water was filled to a depth of 5.5 cm in each container. In high-density containers, each larva was kept separately in a cell (6 × 6 × 6.5 cm) partitioned by transparent plates with holes to prevent individuals from direct physical interactions, but to allow transparent sight and water movement within them. A cell of the same size was set in the middle of each container of the low-density containers, and a single larva was kept in it.

The larvae were observed every day to check if they had reached the feeding stage (stage 44). Each container was cleaned three times a week and refilled with fresh water. Snout-vent length and head width were measured at the start of the experiment (hatchlings) and on the day when they reached the feeding stage.

Size (snout-vent length and head width) of hatchlings (stage 41), length of pre-feeding stage (stages 41 to 43) period, and pre-feeding stage growth rate (growths of snout-vent length and head width per day, and relative growth of head width to snout-vent length) were compared between the Erimo and the Bankei populations. The relative increase of head width to snout-vent length during the pre-feeding stage was calculated after the values were log-transformed. That is, relative growth of head width to snout-vent length was calculated as [{log (head width at feeding stage) - log (head width in hatchlings)} / {log (snout-vent length at feeding stage) - log (snout-vent length in hatchlings)}]. The log-transformation was applied in order to correct the allometric relationship between the two different measurements. To test the effects of population and density on these parameters, two-factor-ANOVA was conducted.

RESULTS

Field observations

Eighteen cannibal/victim pairs were observed at the pond in Erimo. One victim had been caught by its tail, and its head was stuck in its cannibal's mouth, and two other victims were not able to be pulled from the cannibal's oral cavity. These three pairs were excluded from the following analysis. The developmental stages ranged from stages 46 to 52, 42 to 47, and 41 to 51 for the cannibals, victims, and randomly collected larvae, respectively.

Seven out of 15 victims and 0 out of 15 cannibals were at pre-feeding stage (stages 42 and 43), and 4 out of 30 randomly collected larvae were at pre-feeding stage (stages 41 and 43). The proportion of the number of pre-feeding stage larvae to the feeding stage larvae was significantly greater for the victims than for the randomly collected larvae ($\chi^2 = 14.42$, df = 1, $P < 0.01$).

Snout-vent length of the cannibals was larger than that of randomly collected individuals ($t = -5.85$, df = 43, $P < 0.01$). Whereas that of victims was not significantly different from that of randomly collected larvae ($t = 1.15$, df = 43, $P = 0.26$). Relative size of head width to snout-vent length is compared between the victims and the randomly collected individuals within the range of victims' stages (42 to 47) (Fig. 1A). ANCOVA revealed that head width after removing the effect of snout-vent length is smaller in victims than in randomly collected individuals (heterogeneity of slopes, $F = 0.69$, df = 1,33, $P = 0.41$; elevation, $F = 20.30$, df = 1,34, $P < 0.01$). Figure 1B shows the comparison of relative size of head width to snout-vent length between the cannibals and the randomly collected individuals within the range of cannibals' stages (46 to 52). ANCOVA revealed that there is no significant difference between cannibals and randomly collected individuals in head width after removing the effect of snout-vent length (heterogeneity of slopes, F = 0.04, df = 1,34, P = 0.84; elevation, F = 2.37, df = 1,34, P = 0.13).

Laboratory experiments

The average snout-vent length of the hatchlings (stage 41) from the Erimo population was significantly larger (by ca. 10%) than that from the Bankei population, but their head widths did not differ between the populations (Table 1). All larvae from both populations reached the feeding stage (stage 44) by the 7th day after hatching. Both snout-vent length and head width growths during pre-feeding stage were significantly larger (by more than 40%) in larvae originating from the Erimo population than in those from the Bankei population (Table 1). Relative head width in the pre-feeding stage increased faster in the larvae of the Erimo population than in those of the Bankei population (Table 1). Two-way ANOVA showed no effect of density or interaction on any of the size and growth parameters (Table 1).

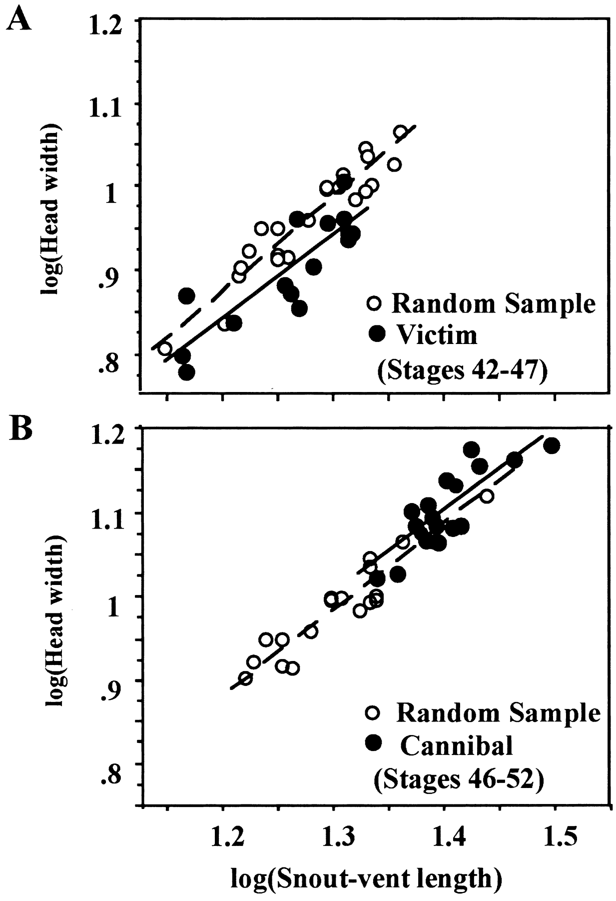

Fig. 1. Comparison of relative head width to snout-vent length in larval *Hynobius retardatus* from a natural pond of Erimo population. (A) Comparison between victims and randomly collected larvae; (B) Comparison between cannibals and randomly collected larvae. Comparisons were made between larvae within the same ranges of developmental stages.

DISCUSSION

Observations in the field revealed that pre-feeding stage larvae in the pond were fed more heavily by their conspecifics compared to feeding stage larvae. The data also showed that larvae with proportionally smaller head widths are more vulnerable to cannibalism than those with larger heads. Although the differences of absolute head widths rather than proportional might be more important in determining the outcome of cannibalism as shown in Ohdachi (1994) and Kusano *et al.* (1985), the results support the hypothesis that being the most vulnerable stage to cannibalism, pre-feeding stage larvae with larger head will reduce the risk of being cannibalized. The hypothesis that wider head increases the possibility of preying on smaller conspecifics was not supported in the present

Table 1. Results of two-way ANOVA on the growth parameters (mean ± SD) of larvae from two populations under high- and low-density treatments

Population & Treatment or Sources of Variation	Size of hatchlings (stage 41)		Pre-feeding stage (stages 41–43) growth rate			Sample size
	Snout-vent length (mm)	Head width (mm)	Snout-vent length (mm/7days)	Head width (mm/7days)	*Relative growth of head width to snout-vent length	
Erimo population						
High-density treatment	11.94 ± 0.62	3.98 ± 0.43	3.00 ± 0.92	1.73 ± 0.52	1.69 ± 0.40	N = 24
Low-density treatment	12.29 ± 0.56	3.98 ± 0.42	2.84 ± 0.76	1.70 ± 0.61	1.74 ± 0.47	N = 16
Bankei population						
High-density treatment	11.05 ± 0.81	3.83 ± 0.42	1.68 ± 0.69	0.68 ± 0.33	1.19 ± 0.49	N = 26
Low-density treatment	11.10 ± 0.73	3.84 ± 0.40	1.60 ± 0.66	0.56 ± 0.31	1.23 ± 1.29	N = 18
Results of two-way ANOVA						
Population	df=1,80 F=44.73 P<0.01	df=1,80 F=2.35 P=0.13	df=1,80 F=55.54 P<0.01	df=1,80 F=120.67 P<0.01	df=1,80 F=9.75 P<0.01	
Density	df=1,80 F= 1.73 P=0.19	df=1,80 F=0.002 P=0.96	df=1,80 F= 0.48 P=0.49	df=1,80 F= 0.60 P=0.44	df=1,80 F=0.09 P=0.77	
Pop. × Dens.	df=1,80 F= 0.98 P=0.32	df=1,80 F=0.001 P=0.97	df=1,80 F= 0.05 P=0.83	df=1,80 F= 0.19 P=0.66	df=1,80 F=0.001 P=0.98	

* Relative growth of head width to snout-vent length is calculated after log-transformation. See text for detailed formula.

result because there is no difference in proportional head size between cannibals and randomly sampled individuals. However, it is true that the absolute body size of cannibals measured as their snout-vent length was larger than that of randomly sampled larvae. Whether large individuals tend to cannibalize others or cannibalistic individuals tend to become larger than the rest of the conspecifics is unknown.

Relative head width increased in a faster rate as well as absolute head width and snout-vent length during the pre-feeding stage in larvae of the cannibalistic Erimo population than in larvae of the non-cannibalistic Bankei population. This result is consistent with my prediction that faster growth in head size is favored in a population with frequent cannibalism. Erimo population has larger egg size than Bankei population (unpublished data), which might be causing the greater overall growth during the pre-feeding stage in Erimo population, although egg size is most likely determined by combinations of factors such as altitude (Takahashi and Iwasawa, 1988) and breeding habitat (Petranka *et al.*, 1987; Woodward, 1982).

Alternative way to reduce the risk of being cannibalized is to produce larger hatchlings and/or shorten the duration of the pre-feeding stage period. Kaplan (1980) reported that *Ambystoma tigrinum* produces larger hatchlings and shortens the length of the pre-feeding stage by laying large eggs. The size of hatchlings cannot be interpreted as an adaptation to minimize cannibalism in this species, *H. retardatus*, because although the snout-vent length was significantly larger in hatchlings from the Erimo population, the head width of hatchlings which is more critical for avoiding cannibalism was not significantly different between these two populations. The length of the pre-feeding stage period was 7 days in larvae from both populations at the temperature of 17°C. The larvae were observed daily, and the range of the length of period was 6 to 7 days, indicating a highly synchronized development in the two populations despite having different egg sizes. In *A. tigrinum*, the duration from hatching to start feeding varies as much as 1 month among the embryos of different females (Kaplan, 1980).

I found no effect of rearing density on any of the pre-feeding stage growth parameters. In my previous studies (Nishihara 1996a, b), I showed that there is density-induced phenotypic plasticity in overall growth pattern of larvae (including both pre-feeding and feeding stages) from both Erimo and Bankei populations. These results indicate that the effect of rearing density on growth and morphology of the larvae appears during the feeding stage in this species. In addition, the significant population effect on growth parameters of pre-feeding stage regardless of the rearing density indicates that genetic variation of these larval traits exists among local populations of this species.

The results from the field observation suggest that the larval head morphology and growth in Erimo population are currently under selective pressure of being cannibalized, but this does not necessarily mean that these traits are under directional selection. Possible costs of cannibalism have been known such as risk of injury or death, risk of eating a relative (reviewed in Crump, 1992), or risk of consuming pathogens from victims (Pfennig *et al.*, 1991). If any of these costs is associated with the cannibalistic characters of the larvae, they could be under balancing selection. To draw conclusions to what kind of selection the current population is experiencing, more information on these possible costs should be obtained.

Previous experiments which examined the factors inducing the cannibal morphs of ambystomatid salamanders (Collins and Cheek, 1983; Pfennig and Collins, 1993; Walls *et al.*, 1993) had been terminated and measured when the larvae were at some stage near metamorphosis. The growth pattern of the head morphology throughout the pre-feeding and feeding stages should be considered separately. The results of the present study emphasize the significance of acquiring large head size during pre-feeding stages, i.e. the stages most vulnerable to cannibalism, supporting the hypothesis that a wide head is an adaptation against frequent cannibalism in larval *H. retardatus*.

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