High Density Induces a Large Head in Larval *Hynobius retardatus* from a Low Density Population

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Abstract: Cannibalism is common among larval *Hynobius retardatus*, especially when density is high. Possessing a large head should be advantageous under such conditions. Relative head size grew larger when larvae were reared under high density conditions even in larvae originating from a low density population.

Key words: *Hynobius retardatus*; Cannibalism; Salamander; Head morphology; Head growth

Larger head and mouth widths are advantageous among cannibalistic larval salamanders, since their predation is gape-limited (Kusano et al., 1985; Ohdachi, 1994). Head width of *Hynobius retardatus* larvae from the Erimo population where egg density is high and cannibalism frequently occurs was larger than in those from the Bankei population (Nishihara, 1996). This supports the hypothesis that higher density induces a higher rate of cannibalism and, consequently, a larger head becomes advantageous.

Cannibal morphs in *Ambystoma* larvae characterized by a disproportionately large head (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). In *H. retardatus*, Nishihara (1996) reported that the larval head grew larger when reared under high density conditions even without any actual cannibalism or cannibalistic interactions. The experiment showed that head size in this species is determined partly by density-induced phenotypic plasticity. However, the experiment was conducted only on larvae from the cannibalistic Erimo population, and did not examine whether such plasticity exists in individuals of the non-cannibalistic Bankei population.

In this paper, I report the results of the high- and low-density experiments conducted on the larvae from the Bankei population. I also estimate the egg densities of the two populations from 1993 to 1995.

**MATERIALS AND METHODS**

Larvae of the Bankei population were reared under high- and low-density conditions. The experimental design, containers used for treatments, and feeding protocol were the same as those of a previous study (Nishihara, 1996). The containers were placed on the same rack in the same room as that for the Erimo population (42°41'N, 141°08'E; 200 m elevation). Eggs from nine clutches were collected within 24 h of oviposition from a pond near Horomi Pass of Bankei (43°02'N, 141°18'E; 260 m elevation; Bankei population) on 25 April 1995. They were kept in the laboratory and placed in the high (nine individuals per container) and low density (one individual per container) containers just after hatching. Containers (18 cm × 18 cm × 6.5 cm) were made of transparent acrylic plates (0.5 cm thick). In high-density containers, each larva was kept separately in a cell (6 cm × 6 cm × 6.5 cm) partitioned by transparent plates to prevent direct physical interactions, but which had holes to allow water movement. A cell of the same size was set in the middle of each container of the low-density experiment and a single larva was kept in it. Each container was filled with non-chlorinated water to a depth of 5.5 cm.

Larvae from nine different clutches were assigned to containers in such a way as to minimize sibship effects. For high-density treatment, one hatching from each of nine different clutches was placed randomly into one of nine cells in each container. For low-density treatment, two hatchlings from each of nine different clutches were released into cells of different containers. These experiments were conducted under a photoperiod of 15 h light: 9 h dark and a constant temperature of 17°C. One individual in high-density treatment died during the experiment and was excluded from the analyses. It was replaced by another larva to maintain the original density of the container. The final sample sizes were 26 and 18 for high- and low-density treatments, respectively. From the 8th day after hatching, the larvae were fed three times per week with equal quantities of live *Tubifex*. The unconsumed *Tubifex* were weighed after feeding. Each container was cleaned three times a week and refilled with fresh water.

Snout-vent length (SVL: distance from anterior tip of snout to posterior margin of vent, to the nearest 0.01 mm) and head width (HW: maximum width across the dorsal side of the head, to the nearest 0.01 mm) were measuredAccepted 1 Oct. 1996
every seven days from the start of the experiment (day of hatching) until the 56th day. They were also measured when larvae reached the stage of metamorphosis (stage 66). Thus, ten repeated measurements were conducted per individual. Wet weight (to the nearest 0.01 g) was recorded on the 28th and the 56th day after hatching and at metamorphosis. Since the larval development of H. retardatus is very similar to that of Hynobius nigrescens, developmental stages were determined according to Iwasawa and Yamashita (1991), who describe the normal stages of H. nigrescens.

Eggs laid in the pond of the Bankei population were counted in 1993, 1994, and 1995. Those of the Erimo population were counted in 1994 and 1995. Surface area (square meters) of each pond was calculated as \((\text{length}/2)^2 \times (\text{width}/2) \times \pi\), and the egg density (number of eggs/square meter of the pond) in each year was calculated as an index of larval density.

Statistical analyses were performed using programs implemented in Systat 5.2.1 and Statview II 1.03.

**RESULTS**

The accumulated mass of food residue collected throughout the experimental period did not exceed 0.5% of the total food mass supplied to any one individual, indicating that the food intake of each individual was approximately equivalent. In the high-density treatment, no differences in snout-vent length or head width of individuals reared in different containers were detected by the two-factor repeated measures ANOVA, using the container as the main effect and the date as the repeated measure (SVL, container \times repeated measures, \(F_{18,207} = 0.51, p = 0.95\); effect of container, \(F_{2,23} = 0.15, p = 0.86\); HW, container \times repeated measures \(F_{18,207} = 0.58, p = 0.91\); effect of container, \(F_{2,23} = 0.31, p = 0.74\)). Consequently, individuals subjected to high-density treatment were pooled in subsequent analyses.

Relative head width grew larger in larvae in the high-density treatment than those in the low-density treatment (Fig. 1), although it did not differ at the beginning of the experiment (ANOVA, using SVL as a covariate, heterogeneity of slopes \(F_{1,40} = 0.37, p = 0.54\); effect of density, \(F_{1,41} = 0.16, p = 0.69\)). Differences in growth pattern of snout-vent length and head width between treatments were tested by two factor repeated measures ANOVA using density as the main effect and the date as the repeated measure. The interaction between density and repeated measures and the effect of density on SVL were not significant (density \times repeated measures, \(F_{9,378} = 1.26, p = 0.26\); effect of density, \(F_{1,42} = 0.01, p = 0.92\)), but interaction between density and repeated measures on HW was detected (density \times repeated measures, \(F_{9,378} = 3.09, p < 0.01\)). The difference between the treatments declined at metamorphosis (ANCOVA, using snout-vent length as a covariate, heterogeneity of slopes \(F_{1,40} = 0.17, p = 0.68\); effect of density, \(F_{1,41} = 3.54, p = 0.06\)).

Developmental rate (developmental stages) did not differ between the treatments until the 56th day. Days until metamorphosis ranged from 74 to 97, and was shorter in high-density treatment than in low-density treatment (Mann-Whitney U-test, \(z = -2.41, p = 0.016\)). There were no differences in wet weight on the 28th and 56th day after hatching, and at metamorphosis between high- and low-density treatments (28th day after hatching, high-density treatment, \(0.20 \pm 0.04\) g; low-density treatment, \(0.20 \pm 0.03\) g; 56th day after hatching, high-density treatment, \(0.50 \pm 0.06\) g; low-density treatment, \(0.49 \pm 0.04\) g; at metamorphosis, high-density treatment, \(0.51 \pm 0.05\) g; low-density treatment, \(0.52 \pm 0.07\) g; \(t_{38} = 0.72, p = 0.48\); at metamorphosis, high-density treatment, \(0.51 \pm 0.05\) g; low-density treatment, \(0.52 \pm 0.07\) g; \(t_{38} = 0.37, p = 0.72\).

Egg density of the Bankei population was 62/m², 55/m², and 47/m², in 1993, 1994, and 1995, respectively, and that of the Erimo population was 382/m² in 1994 and 481/m² in 1995 (the
data in 1994 were formerly reported in Nishihara, 1996).

DISCUSSION

Larval heads grew larger in high-density treatment than in low-density treatment for both the Erimo population (Nishihara, 1996) and the Bankei population (this study), indicating that the individuals from the Bankei population had the same density-induced phenotypic plasticity of larval head size as those from the Erimo population. Although egg densities were consistently low in the Bankei population during the three years from 1993 to 1995, the individuals of the Bankei population might have maintained their sensitivity to density for those years of high density in a longer time span. Heterogeneity of environment and cost of plasticity are important factors determining the advantage of phenotypic plasticity over a genetically determined phenotype (Bamber and Henderson, 1988). These data should be obtained for further discussion of the adaptive significance of plasticity in larval morphology.

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