Allometric dimorphism in the larvae of the salamander *Hynobius retardatus*

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*Hynobius retardatus* has a head-width plasticity that is functional in cannibalism and preying on large creatures. Cannibalistic specialization in *H. retardatus* appears to a lesser degree than in those species that have clearly dimorphic morphs. To examine the degree of cannibalistic specialization and intraspecific phenotypic variation, we investigated the allometric relationship between the head and body in the larvae of *H. retardatus* in a single pond. In addition, the effect of cannibalism on the determination of morphology was examined. A field survey revealed the existence of allometric dimorphism and the occurrence of large-headed large larvae. However, we did not observe a bimodal size distribution that indicates a split in the population. A laboratory experiment revealed that the development of such large-headed large larvae was inducible by actual cannibalism and/or additional tactile cues under high-density conditions. We deduce that the allometric dimorphism in the larvae of *H. retardatus* in the wild is caused by actual cannibalism. The cannibalistic phenotypic variation in *H. retardatus* would be characterized by allometric dimorphism without a bimodal size-frequency distribution.

**Key words**: cannibalism, cannibal morph, phenotypic plasticity, intraspecific competition, intraguild predation, salamander, *Hynobius*.

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INTRODUCTION

Some amphibian larvae exhibit two alternative morphs, called “cannibal morph” and “typical morph” or “carnivore” and “omnivore” and are intriguing examples of adaptive phenotypic plasticity (Collins & Cheek 1983, Pfennig 1990, Roff 1997, Hall 1999). However, the dichotomy of typical morph and the alternative cannibalistic morph is not always clear, suggesting that the range of cannibalistic plasticity is considerably variable among species (Nyman et al. 1993). This interspecific difference in cannibalistic phenotypic plasticity illustrates the relationship between ecological factors and the evolution of phenotypic plasticity. The dichotomy of the phenotype is exhibited in multiple forms such as the allometric relationship between head and body sizes (Lanoo & Bachmann 1984, Pfennig 1990), the dental structure (Orthon 1954, Pedersen 1991), and the size-frequency distribution of larvae in a single pond (Lanoo & Bachmann 1984, Pfennig 1990). The cannibal morph of Ambystoma tigrinum and the carnivore of Scaphiopus toads are radically different from their typical counterparts in that they have an exaggerated head size, enlarged mouth structure (Orthon 1954, Lanoo & Bachmann 1984, Pfennig 1990, Pedersen 1991), and are separated from typical larvae by a bimodal size-frequency distribution (Lanoo & Bachmann 1984, Pfennig 1990).

Phenotypic plasticity sometimes gives rise to a bi- or poly-modal discontinuous variation, that is called “polyphenism” (West-Eberhard 1989). While the larval morphology of A. tigrinum and Scaphiopus toads are discontinuous and thus, clearly show “polyphenism” (Lanoo & Bachmann 1984, Pfennig 1990), the morphological variations in other species are moderate and not proved to be discontinuous (A. annulatum: Nyman et al. 1993; A. macrodactylum: Walls et al. 1993; Hynobius retardatus: Nishihara 1996). Therefore, polyphenic feature of these species are “moderate”. The degree of intraspecific dichotomy (i.e., cannibal morph and typical morph) may differ between these moderately cannibalistic species and clearly dimorphic species in some aspects. However, the within-pond phenotypic variation in the allometric relationship between the head and body and the size-frequency distribution in moderately cannibalistic species is rarely investigated (Nyman et al. 1993, Nishihara 1996).

The main aim of our study is to understand the intraspecific phenotypic variation in the Japanese salamander H. retardatus. Crowding conditions induce widening of the larval head (Nishihara 1996, Kohmatsu 2001, Michimae & Wakahara 2001). The characteristic of H. retardatus is that “density-induced morphology” occurs in most, if not all, individuals and as a result, larval average head width increases without producing any particular cannibal morphs (Nishihara 1996). The morphological variation in H. retardatus seems continuous (Nishihara 1996); however, the views on the existence of morphological dimorphism in this species are inconsistent between the studies (Wakahara 1995, Nishihara 1996, Kohmatsu et al. 2001), and the within-pond phenotypic variation is poorly understood. To our knowledge, no statistical analysis has been conducted to verify the morphological dimorphism in H. retardatus, nor in other cannibalistic amphibian larvae. In order
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to verify allometric dimorphism in larval populations, we applied a morphometric
analysis that is called the EBERHARD & GUTIERREZ model (“E & G model”; EBERHARD &
GUTIERREZ 1991, KOTIAHO & TOMKINS 2002). In parallel with the E & G model
analysis, the size-frequency distributions of the natural pond larvae were investiga-
ted to explore the phenotypic divergence within the population.

While focusing on the within-pond phenotypic variation, it is of great interest
to examine how the fate of an individual larva is determined i.e., whether it will be
cannibalistic or not. Several factors are known to affect the occurrence of the wide-
headed larvae of H. retardatus; these include larval density (NISHIHARA 1996), kin-
ship (MICHIWA & WAKAHARA 2001), chemical cues (NISHIHARA 1996), visual cues of
conspecifics (KOHMATSU 2001), and tactile cues of Rana pirica tadpoles (MICHIWA &
WAKAHARA 2002, MICHIWA et al. 2005). However, which individuals attain exaggerat-
ed head morphology remains unknown. WAKAHARA (1995) reported that the larvae with
large heads were found only in the tanks where actual cannibalism had occurred and
suggested that actual cannibalism had caused the morphogenesis. However, thus far,
the causal relationship between cannibalism and morphogenesis remains unknown.
Therefore, we explored the relationship between cannibalism and morphogenesis by
artificially provisioning conspecific larvae to the experimental larvae.

MATERIALS AND METHODS

Study sites

Two natural breeding ponds of H. retardatus in Hokkaido were used for the field survey

Laboratory Study I. Effect of diet on morphogenesis

It is already known that the density effect (chemical and visual cues) can induce head
enlargement without cannibalism in H. retardatus (NISHIHARA 1996, MICHIWA & WAKAHARA
2001). We applied the same experimental design as these previous studies that induce head
enlargement by chemical and visual cues (Fig. 1, NISHIHARA 1996, MICHIWA & WAKAHARA 2001).
The density stimulation in our experiment (1 larva/16 cm²) is intermediate compared with the
previous studies (NISHIHARA 1996: 1 larva/36 cm²; KOHMATSU 2001: 1 larva/36 cm²; MICHIWA &
WAKAHARA 2001: 1 larva/9 cm²). Therefore, our experiment can effectively reproduce “density-
this design, we induced “density-induced morphogenesis” (see Results) and examined wheth-
er actual cannibalism cause an additional morphogenesis by feeding conspecific larvae to the
“density-induced” larvae (Fig. 1, high-density treatment). In parallel with high-density treatment,
the sole effect of cannibalism was tested in a solitary condition (Fig. 1, low-density treatment)
where “density-induced morphogenesis” does not occur (NISHIHARA 1996).

Naturally fertilized egg sacs were collected from the Erimo population and allocated
to these experiments. Larvae belonging to two families were mixed and used. These were
hatched and reared at 20 °C and under 15L:9D conditions. They were fed either conspecific
hatchling(s) (total length 20 mm, approximately) or Tubifex (sewage worm). Larval sizes at
the beginning of the experiment were 18.8 ± 0.29, 18.8 ± 0.41, 18.8 ± 0.44, 18.7 ± 0.61 in
total length (mm, ± SD), for treatment of high-density-Tubifex, high-density-hatchling, low-
density-Tubifex, low-density-hatchling respectively and they did not differ between treatments
(ANOVA, $F_{3,35} = 0.12, P = 0.95$). After the prefeeding growth by egg yolk, the larvae begin feeding at 7 days of age (Nishihara 1996); therefore, they were fed on the 7th, 10th, and 13th days after hatching. On the 14th day, the largest head width and snout-vent length (SVL) were measured using a digital caliper to the nearest 0.01 mm. The larvae were fed the maximum amount of diet that can be consumed within an hour (1-2 larva(e), 10-15 mg Tubifex).

In the high-density treatment, the larvae were reared together (1 larva per 16 cm$^2$) but separated in cells (4 × 4 cm) partitioned by perforated (diameter; 1 mm; 12 holes) and transparent acrylic boards. Actual cannibalism was inhibited by the partitions unless larvae were fed conspecific hatchling(s) artificially (Fig. 1). Since the density effect alone can produce “density induced morphology” in this design (Nishihara 1996, Kohmatsu 2001, see Results), an additional effect of larval diet was measured by artificially provisioning hatchlings to the larvae in the high-density condition. Although we controlled the density effect, we could not eliminate the tactile cue from the larvae provided as diet in this experiment. The larvae subjected to a low-density treatment were reared individually in opaque cups (diameter, 3 cm) so that they were exposed to a single stimulation of diet without any density effect (Fig. 1). Eight to 12 larvae were allocated to each experimental group (Fig. 1).

In the statistical analysis, log-transformation was conducted on head width and SVL in order to precisely evaluate allometric relationships and increase statistical power.

In our experimental set-up, the larvae in the middle of the set-up seem to receive stronger chemical and visual stimulations than those along the edge of the set-up. However, the cell location (edge or middle) did not affect the larval morphology (two-way ANCOVA, factors: cell location, density; covariate: ln SVL, $F_{1,12} = 1.03, P = 0.33$). Thus, we pooled the data from all the cells and analyzed them together.

**Field study: Demographic research of natural populations**

Two natural breeding ponds of H. retardatus in Hokkaido were used for the field survey (the Erimo population: Meguro, Erimo, N42°, E143°, and the Bankei population: Bankei, Sap-
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The eggs began hatching at the beginning of May in the Bankei population and in the middle of May in the Erimo population. The first collection was conducted at 1 month after the beginning of hatching, and then, the larvae in the same ponds were collected monthly (the Bankei population: June 2 N = 77, July 1 N = 153, August 9 N = 54, the Erimo population: June 14 N = 67, July 9 N = 63). We did not have 3-month-old larvae in the Erimo population because the larval development was fast in this population, and all larvae had completed metamorphosis by the age of 3 months. Therefore, we have a total of five data sets (the Bankei population aged 1, 2, and 3 months and the Erimo population aged 1 and 2 months). The larvae were collected randomly by using a scoop net and anesthetized by MS222 immediately after collection to avoid the loss of the collected larvae by cannibalism. The largest head width and snout vent length were measured using digital caliper to the nearest 0.01 mm at the laboratory. No overwintering larvae were observed in either population.

**Morphometric analysis**

Cannibalistic morphology is commonly represented by a large body size and a relatively large head width. In particular, the allometric dimorphism in a single pond is the most diagnostic character of the overall phenotypic variation (Pierce et al. 1983, Pfennig 1992). We have no evidence that clarifies whether allometric dimorphism in H. retardatus occurs in a single pond or whether the morphological variation is simply continuous. If cannibalistic dimorphism exists, the larvae of the cannibalistic group have a large body size and a relatively large head size and are thus distinguished from the small-sized and small-headed typical larvae (Pierce et al. 1983, Pfennig 1992). Taking into consideration the general phenotypic variation that characterizes the cannibalistic alternatives, we hypothesized that dimorphism is expressed in either of the following two forms: the occurrence of an inflection in the allometry (Fig. 2a) or a change in the intercept of the regressions (Fig. 2b) or both. To clarify the existence of allometric dimorphism, we adopted the E & G model that elucidates the allometric switch point in the scatter plot of trait size and body size.

In summary, the E & G model (Eberhard & Gutierrez 1991) may be defined as follows:

\[
Y = \alpha_0 + \alpha_1 X + \alpha_2 X^2 + \epsilon
\]  

(1)

Here, \(Y\) is the log-transformed trait size (head width), \(X\) is the log-transformed body size (SVL), \(\alpha_i\) is the regression coefficient, and \(\epsilon\) is the error. If \(\alpha_2\) is significantly different from zero, the regression is judged to be nonlinear, and the original relationship between the

Fig. 2. — Schematic view of allometric dimorphism and switch point (modified from Eberhard & Gutierrez 1991): a, Change in slope; b, Change in intercept. The dashed line indicates the switch point. The dimorphism patterns in a and b are named “Model 3” and “Model 2,” respectively, by Eberhard & Gutierrez (1991).
head width and body size includes more than one linear relationship. In this first step, the propriety of setting a switch point is examined.

\[ Y = \beta_0 + \beta_1 X + \beta_2 (X - X_0) D + \beta_3 D + \varepsilon \]  

Here \( Y \) and \( X \) are actual measurements of head width and body size, \( X_0 \) is the proposed switch point, \( D = 0 \) if \( X < X_0 \), \( D = 1 \) if \( X \geq X_0 \), \( \beta_i \) is the regression coefficient, and \( \varepsilon \) is the error. The switch point \( X_0 \) can be determined by iterating the \( X_0 \) value that provides the best fit for the equation (highest \( R^2 \)) (2). If \( \beta_3 \) is significantly different from zero, the intercepts of regressions at the switch point are judged to differ at the switch point \( X_0 \) (Fig. 2b). If \( \beta_2 \) is significantly different from zero, the linear slope between the head width and body size changes at the switch point \( X_0 \) (Fig. 2a). If either \( \beta_2 \) or \( \beta_3 \) or both are significant, the relationship between the head width and body size is dimorphic.

There is one difficulty in applying the E & G model to salamander larvae. The relative head size of the larvae changes with larval ontogeny itself (Nishihara 1996). Thus, allometric changes may not be the result of cannibalistic morphogenesis. The experiment conducted in the ponds revealed that large \( H.\ retardatus \) larvae metamorphose earlier than small larvae (Kohmatsu et al. 2001). This implies that large larvae undergo ontogeny faster than small larvae in a single pond. If the large larvae that develop faster possess relatively large heads, it is not possible to distinguish these larvae from the cannibalistic ones by studying the allometric relationship between the head and body. To eliminate this difficulty, we examined the developmental allometry of \( H.\ retardatus \). The details of the method used are provided in the section describing Laboratory Study II.

In addition to the E & G model, the size-frequency distribution was examined by depicting the histograms of SVL and the largest head width of the larvae. It is known that two groups in the bimodal size-frequency distribution correspond to the cannibal morph and the typical morph (Lanoo & Bachtsmann 1984, Pfennig 1990). Therefore, if the population of a single pond has a bimodal size-frequency distribution, it has two dimorphic groups.

**Laboratory Study II. Measurement of developmental allometry**

We measured the growth pattern of head and body throughout the development of the larvae. Naturally fertilized egg sacs were collected from the Erimo population and allocated to this experiment. Fifty-six larvae from seven families were used for the experiments. The larvae were hatched and reared at 20 °C under 15L:9D conditions.

To recreate a condition of natural density, an experimental set-up identical to Laboratory Study I but contains 16 cells was used. As in Laboratory Study I, actual cannibalism was prohibited by the partitions. The largest head width and total length were measured weekly from the time of hatching until the larvae were 7 weeks old. The measurements were conducted by image analysis program “Image J” (NIH) to reduce the disturbance from frequent measurements. The image of the focal larva with a ruler was captured by a digital camera (C3100 ZOOM, Olympus, Tokyo), and then the larval size was calculated from the ruler-larva ratio in “Image J” program. We used the total length as an index of body size in this experiment because of the difficulty of measuring SVL from captured images. The larvae were fed Tubifex (sewage worm) every 3 days from the time they were 7 days old until the end of the experiment. The amount of food provided was the maximum amount that the larvae eat in an hour. The water depth was maintained at 5 cm, and the water was replaced every 3 days. As in Laboratory Study I, the cell-locations (edge, middle) did not cause bias in body and head growth (repeated measures ANOVA, factor: cell-location, repeated measure: age; total length: \( F_{1,54} = 0.09, P = 0.77 \); largest head width: \( F_{1,34} = 0.40, P = 0.54 \)). Thus, we pooled the data from all the cells and analyzed them together.
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RESULTS

Laboratory Study I. Effect of diet on morphogenesis

The scatter plot of head width against body size is shown in Fig. 3. At first, two-way ANOVAs were conducted to analyze the effect of diet and density on head width and body size. Larval head width (ln largest head width) was affected by density and diet (two-way ANOVA, density: $F_{1,35} = 13.6, P < 0.001$; diet: $F_{1,35} = 37.9, P < 0.001$; diet × density: $F_{1,35} = 0.71, P = 0.41$), whereas larval body size (ln SVL) was affected only by diet (two-way ANOVA, density: $F_{1,35} = 0.405, P = 0.53$; diet: $F_{1,35} = 94.0, P < 0.001$; diet × density: $F_{1,35} = 0.004, P = 0.95$). These results show that high-density induces head enlargement without changing body size, supporting the “density-induced morphogenesis” found in previous studies (Nishihara 1996, Kohmatsu 2001).

Since diet affects body size, diet effect on head width was analyzed by ANCOVA using body size (ln SVL) as a covariate. We could not use two-way (diet × density) ANCOVA to analyze the effect of diet, because the interaction terms were significant (diet × density × ln SVL: $F_{1,31} = 4.93, P = 0.034$; diet × density: $F_{1,31} = 4.75, P = 0.037$). In the high-density treatment, the regression slope significantly differed between diets (Fig. 4, ANCOVA, factor: diet, covariate: ln SVL; diet × ln SVL: $F_{1,16} = 5.196, P = 0.037$). In contrast, neither the regression slope nor the intercept differs between diets in the low-density treatment (Fig. 4, ANCOVA, factor: diet, covariate: ln SVL; diet × ln SVL: $F_{1,15} = 0.577, P = 0.46$; diet: $F_{1,15} = 0.472, P = 0.503$).

Field study. Demographic research of natural populations

Among the five data sets, the data on the Bankei population aged 1 and 2 months and the Erimo population aged 1 month passed the first test of the E & G
Fig. 4. — Demographic traits of wild *H. retardatus* larvae: *a*, Bankei larvae aged 1 month; *b*, Bankei larvae aged 2 months; *c*, Bankei larvae aged 3 months; *d*, Erimo larvae aged 1 month; *e*, Erimo larvae aged 2 months. The dashed lines indicate allometric switch points demonstrated by the E & G model. The solid lines indicate the best-fit regressions calculated by the E & G model. Note that the scale for the Bankei larvae aged 1 month is magnified two-fold. Bankei larvae aged 1 month: $Y = 0.433X + 0.366(X – 17.3)D + 0.215$ (best switch point $D^0 = 17.3$, $R^2 = 0.875$), Bankei larvae aged 2 months: $Y = 0.368X + 0.128(X – 20.13)D + 0.875$ (best switch point $D^0 = 17.3$, $R^2 = 0.885$), Bankei larvae aged 3 months: $Y = 0.419X – 0.586$ ($R^2 = 0.93$), Erimo larvae aged 1 month: $Y = 0.613X – 0.225(X – 21)D – 2.09$ (best switch point $D^0 = 21$, $R^2 = 0.94$), Erimo larvae aged 2 months: $Y = 0.455X – 0.719$ ($R^2 = 0.92$).
model that supports the propriety of setting an allometric switch point (the Bankei population aged 1 month, $t_{75} = 2.81$, $P < 0.01$; that aged 2 months, $t_{151} = 3.42$, $P < 0.001$; and that aged 3 months, $t_{52} = 0.35$, $P = 0.73$; the Erimo population aged 1 month, $t_{65} = -2.8$, $P < 0.01$ and that aged 2 months, $t_{61} = 0.23$, $P = 0.82$; t-test for $\alpha_2$, see morphometric analysis in Materials and methods). By applying the second test of the E & G model, the allometric difference was found to be significant across the proposed switch point in all three populations that passed the first test (the Bankei population aged 1 month, $t_{75} = 3.42$, $P < 0.001$; that aged 2 months, $t_{151} = 2.96$, $P < 0.005$; the Erimo population aged 1 month, $t_{65} = -2.73$, $P < 0.01$; t-test for $\beta_2$). The difference in the height of the regression lines was not significant in any of the three populations (the Bankei population aged 1 month, $t_{75} = -0.44$, $P = 0.66$ and that aged 2 months, $t_{151} = 1.75$, $P = 0.082$; the Erimo population aged 1 month, $t_{65} = 1.21$, $P = 0.23$; t-test for $\beta_3$). Therefore, the inflection in the allometry (“Model 3” dimorphism in E & G model, Fig. 2a) was demonstrated in these three populations (Fig. 4). In the Bankei population aged 1 and 2 months, the slope of the regression line increased in the large-sized group. In other words, larger individuals had a larger relative head width in the Bankei population aged 1 and 2 months. Their appearances are shown in Fig. 5. In the Erimo population aged 1 month, the regression slope declined in the large-sized group, demonstrating that larger individuals had a smaller relative head width.

With regard to the size-frequency distributions of body size and head width, no clear bimodality was observed in any of the five data sets. The size-frequency

Fig. 5. — Differences in allometry and body size of large and small larvae in a single pond (Bankei population aged 2 months). Left: large larva with a relatively large head. Right: small larva with a relatively small head. Ventral sides of the larva is shown. Note that the head of the large larva is round-shaped. The magnifications of the two pictures are the same.
Laboratory Study II. Developmental allometry and cannibalistic morphology

The pattern of the scatter plots revealed that head width relative to the body size gradually decreased with larval development (Fig. 6). To describe the reduction in the slope mathematically, the scatter plots of the data on larvae at each age were pooled together and fitted to a binomial curve after log-transformation. The regression was significant, and the regression coefficient of the binomial term was negative (regression, $F_{2,445} = 3308, P < 0.001$; binomial term, $t_{446} = -29.2, P < 0.001$, $Y = -1.17X^2 + 9.01X - 15.24, R^2 = 0.948, X = \ln$ total length, $Y = \ln$ largest head width), indicating that the slope was decreasing. The observed curve suggests that the larvae developing faster are not isometric with the less developed small larvae.
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They are large in size but have a smaller relative head width than the less developed small larvae. Since cannibalistic larvae possess large size and a large relative head width (Lanoo & Bachmann 1984, Pfennig 1990, Wakahara 1995), there is no difficulty in distinguishing the larvae with cannibalistic morphology from the large larvae that develop faster.

**DISCUSSION**

*Effect of diet on morphogenesis*

In our experiment, the density effect (chemical and visual cues) without a diet effect induced head enlargement but did not change larval body size, reproducing “density-induced morphology” that has been observed in previous studies (Nishihara 1996, Kohmatsu 2001, Michimae & Wakahara 2001). In the high-density treatment, those larvae fed with conspecifics not only grew larger in body size and head width, but also exhibited an increase in the regression slope thus expressing a wide-headed morphology, in comparison with those fed *Tubifex* (Fig. 3). We fed conspecifics to the density-induced larvae that had been destined to express density-induced morphology. Therefore, it is suggested that those larvae that were fed conspecifics exhibited an additional morphogenesis on top of the “density-induced morphology” (Nishihara 1996, Kohmatsu 2001, Michimae & Wakahara 2001). This large-headed morphology with large body size gains a considerable advantage over “density-induced larvae” that have not succeeded in cannibalism. Previous studies focused strongly on the variation within “density-induced larvae” (Kohmatsu 2001, Michimae & Wakahara 2001); however, the ecological importance of diet-induced morphology should be included to predict the outcome of larval competition. However, it should be noted that, unfortunately, we could not eliminate the possibility that tactile cues from the larvae that were provided as diet may affect the morphogenesis (Hoffman & Pfennig 1999). The tactile cue may play an important role; the dissociation of these cues needs further investigation.

The diet-induced morphogenesis occurred only under high-density conditions. It is suggested that density stimulation is necessary in cannibalism-induced morphogenesis. From the ecological viewpoint, the possession of a large head is more beneficial when larvae have sufficient potential prey (i.e., conspecifics) around them (Maret & Colins 1994).

Larvae that fed on conspecifics grew larger than those fed on *Tubifex* in Laboratory Study I. Maret & Colins (1994) have argued that the within-pond relative body size is a key determinant in the morphogenesis of _A. tigrinum_. It is possible that the rapid growth induced by cannibalism ensures the greater body size of the larvae that succeed in cannibalism and induces further morphogenesis in _H. retardatus_.

Allometric dimorphism in _H. retardatus_

The E & G model detected allometric dimorphism in the natural _H. retardatus_ population. In the Bankei population aged 1 and 2 months, the larger larvae had a greater relative head width (i.e., the regression slope increased in the larger larvae);
this trait indicates cannibalistic morphology. Previous studies on *H. retardatus* have only tentatively defined “morphs” and lacked an objective view in dealing with pheno-

typic variations (KohMastu et al. 2001, Michimae & Wakahara 2001). The E & G

model enabled us to define two morphologically different groups quantitatively. There

arises the question of how these cannibalistic larvae came into existence. We propose

that diet-induced morphogenesis observed in Laboratory Study I is a very important

factor in determining a cannibalistic tendency in these larvae. Actual cannibalism

with a tactile cue caused a rapid growth and an increase in the regression slope in

Laboratory Study I. Since larvae in the same pond are exposed to similar chemical

and visual density cues, we speculate that the larvae that succeeded in cannibalism

had developed into morphologically different larvae. At present, the behavioural char-

acteristics of the cannibalistic tendency in these larvae remain unclear. Studies on

stomach contents and diet selection of these two groups are essential for revealing

the niche differentiation of the two groups based on allometric patterns.

In contrast to the Bankei population aged 1 and 2 months, the Erimo popu-

lation aged 1 month demonstrated an opposite allometric pattern. We consider that

this can be attributed to the rapid development of the larvae in this population. In

*H. retardatus*, the developmental allometry predicts a decline in the regression slope

(Fig. 6). In other words, the relative head width of larvae gradually decreases with

individual development. The rapid development and wide developmental ranges

of the Erimo larvae may have caused the expression of developmental allometry in

the larvae aged 1 month. Similarly, extended larval developmental stages may have

masked cannibalistic morphology in the Bankei population aged 3 months. The dis-

appearance of allometric change in the Erimo population aged 2 months could be a

result of the metamorphosis of part of the larval population. In summary, the within-

pond cannibalistic allometric dimorphism was observed in the early period of develop-

ment; however, cannibalistic allometric dimorphism did not last long and finally

disappeared, probably with the expanded range of development. In this regard, can-

nibalistic specialization in *H. retardatus* is clearly different from that in *A. tigrinum*

that demonstrates cannibalistic dimorphism even at metamorphosis (Rose & Ar-

менtroat 1976). Cannibalistic specialization in *H. retardatus* is limited to only part of the

life history of the larvae.

**Size-structure of *H. retardatus* in wild populations**

Taking into consideration all observations, although slight dimorphic distribution

was observed in the Bankei population aged 1 month, we conclude that it is

impossible to distinguish the morphs based on the size-frequency distribution in

*H. retardatus*; additionally, the clear bimodality observed in *A. tigrinum* (Lanoo &

Bachmann 1984) or the *Scaphiopus* toad (Pfennig 1990) is absent in this species.

Our field survey contradicts Wakahara’s observation (1995) of larval size bimo-

dality in laboratory tanks. This could be a “giants” effect (Le Cren 1992, Claessen

et al. 2000, Wakano 2004) that may be explained as follows. Where equally sized

larvae grow together; a few larvae that succeed in cannibalism at early stages attain

an overwhelmingly large body size. Subsequently, the larvae that possess large body

and mouth size repeat cannibalisms easily and then form an exceptionally large


group described by Wakahara (1995) could be “giants” in an extraordinarily simple

laboratory environment and is not consistent with what occurs in nature.
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**Cannibalistic specialization in H. retardatus**

The cannibalistic morphology in *H. retardatus* is different from the morphology of typical larvae in terms of the allometric relationship between head width and body size, as shown in Fig. 2a; however, they cannot be differentiated by a bimodal size-frequency distribution, as shown in Fig. 2b. In comparison with clearly dimorphic species, the cannibalistic morphology of *H. retardatus* is less specialized, as determined by a monomodal size-frequency distribution (this study) and a homogeneous teeth structure (NISHIHARA 1996). If the larvae in a single pond are separated into two distinct classes by their size, these groups should be selected differently on the basis of the opportunity to cannibalize smaller individuals or to be cannibalized by larger individuals. In that case, further specialization of highly cannibalistic traits such as the exaggerated teeth structure in *A. tigrinum* (LANOO & BACHMANN 1984, PEDERSEN 1991) or the keratinized beak in *Scaphiopus* tadpoles (ORTHON 1954, PFENNING 1990) may evolve, which results in bimodal “Model 2” dimorphism (Fig. 2b). The absence of a bimodal size-frequency distribution in wild *H. retardatus* would limit the degree of cannibalistic specialization. Comparative studies on the demographic structures of other species are required to understand the evolution of cannibalistic plasticity, its degree, and polyphenism in amphibian larvae.

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**REFERENCES**


