Behav Ecol Sociobiol (2001) 50:339–345
DOI 10.1007/s002650100368

ORIGINAL ARTICLE

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Factors which affect the occurrence of cannibalism and the broad-headed "cannibal" morph in larvae of the salamander *Hynobius retardatus*

Received: 20 September 2000 / Revised: 9 April 2001 / Accepted: 2 April 2001 / Published online: 24 May 2001
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Abstract Cannibalism in amphibian larvae may be suppressed among siblings in comparison to distant relatives or nonkin, even when a "cannibal" morph that can consume conspecifics shows adaptive advantages. Two experimental studies were undertaken to analyze factors which affect the frequency of cannibalism and the occurrence of the broad-headed "cannibal" morph in larvae of the salamander *Hynobius retardatus*. The first experiment investigated whether or not the broad-headed "cannibal" morph is only induced after performing cannibalism. Because the broad-headed "cannibal" morph was observed in larval populations that showed no cannibalism, it was concluded that the "cannibal" morph could be induced without actual cannibalism. Second, possible factors affecting the occurrence of the broad-headed "cannibal" morph were analyzed with respect to the density of larvae, level of food supply, and kinship among larvae, alone or in combination. Appearance of the broad-headed "cannibal" morph was affected by interactive effects of density×kinship: although the morph was induced significantly more often at a higher density if the conspecifics were distantly or not related, it was strongly suppressed even at high density if larval kinship was very close or among siblings. In contrast, the frequency of cannibalism was independently affected by larval density, level of food supply, and kinship among larvae: it was significantly larger at high than at low larval densities, at low than at high food supply, and in mixed-sibling groups than in pure sibships. These results suggest that the "cannibal" morphs in *H. retardatus* are induced even without cannibalism at a high density of conspecifics in mixed-sibling groups, but the occurrence of this morph, which continuously consumes conspecifics, is suppressed among siblings.

Communicated by M. Elgar

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Keywords Cannibalism · Cannibal morph · Kin recognition · Salamander · Inclusive fitness

Introduction

Cannibalism is taxonomically widespread in almost all animals phyla (Elgar and Crespi 1992). In amphibians, larval-larval cannibalism is its most frequent form (Elgar and Crespi 1992). Many studies have suggested that cannibals show faster growth or developmental rates, larger size, higher survivorship, or enhanced reproduction compared with typicals (Fox 1975; Polis 1981). However, although conspecifics are assumed to provide a better balance of nutrients than heterospecific foods (Crump 1986, 1990; Meffe and Crump 1987), cannibalism is limited to certain specific species even in amphibians. The rarity of cannibalism suggests that it must carry considerable fitness costs (Elgar and Crespi 1992). Several fitness costs of cannibalism have been proposed. The first is that cannibals may diminish the indirect component of their inclusive fitness by killing relatives (Hamilton 1964; Waldman 1988; Walls and Roudebush 1991). Although cannibals in *Ambystoma tigrinum* larvae are primarily induced by an increased density of conspecifics in both laboratory experiments (e.g., Collins and Cheek 1983) and in drying ponds in the field (Lannoo and Bachmann 1984),, larvae were more likely to cannibalize distant relatives than close kin. The timing of the cannibal's initial expression is also influenced by the kinship environment (Pfennig and Collins 1993). In spadefoot toad toadpoles (*Spea bombifrons*, *S. multiplicata*), the occurrence of carnivores is not only affected by the density of larvae but also by kinship environment (Pfennig and Frankino 1997), and omnivores aggregate with conspecifics while carnivores are solitary (Pfennig et al. 1993). The second risk of cannibalism is that cannibals may acquire pathogens from conspecifics because of greater genetic similarity among conspecifics than heterospecifics (Pfennig et al. 1998). The third cost is the possibility of injury or even death from retaliation by the proposed victim (Elgar and Crespi 1992).

In *Hynobius retardatus*, the broad-headed “cannibal” morph continuously consumes conspecifics once the morph has become established, whereas typicals ordinarily do not cannibalize (Wakahara 1995). At present, however, it is not clear. in comparison to *A. tigrinum*, what factors induce the occurrence of the broad-headed “cannibal” morph in *H. retardatus*. A few studies with *H. retardatus* have suggested that the broad-headed “cannibal” morph simply occurs as a consequenceof the cannibalism (Wakahara 1995, 1997) or it is induced by a higher density of conspecifics without any actual cannibalism or direct physical interaction (Nishihara 1996). Furthermore, no study has reported whether or not the occurrence of the broad-headed “cannibal” morph is influenced by kinship among larvae.

In this study, we examined whether or not the broad-headed “cannibal” morph is induced as a result of cannibalism. Furthermore, we hypothesized that the broad-headed “cannibal” morph and cannibalism in larvae of *H. retardatus* would be suppressed among siblings in comparison to a situation with distant relatives or no kin, because kinship theory predicts that animals modify their behavior toward conspecifics according to their genetic relatedness (Hamilton 1964). We also examined possible relationships between the occurrence of the broad-headed “cannibal” morph or the frequency of cannibalism and three environmental factors: larval density, level of food supply, and kinship among siblings. As several broad-headed “cannibals” are induced in a tank in *H. retardatus* (Wakahara 1995), we could examine accurately the occurrence rate of broad-headed “cannibal” morphs induced in different environmental conditions. The present study supports the hypothesis that larvae of *H. retardatus* recognize kinship (Wakahara 1997), avoid killing siblings, and increase their inclusive fitness.

Methods

Animals

Fertilized eggs of *H. retardatus* were collected from five ponds located in the vicinity of Sapporo (Bankei 1, Bankei 2, Maruyama, Nopporo, and Teine), two ponds in the vicinity of Otaru (Okusawa 1 and Okusawa 2) and one pond in the vicinity of Erimo, Hokkaido, Japan, during the breeding season (from early April to late May) in 2000. All ponds except Bankei 1 and Bankei 2 are located far apart from each other so that there are no genetic interactions among them. Three or four egg clutches were collected from each pond and placed separately in different large plastic tanks (30×25×17.5 cm) filled with 5 l of dechlorinated tapwater at 4°C in a laboratory until use. Hatching day of embryos and/or developmental stages of larvae were controlled by placing the embryos or larvae either at 4°C or at room temperature (Arai and Wakahara 1993). After hatching, the larvae were fed live freshwater oligochaetes (*Tubifex)*. The rearing water was exchanged every 2 or 3 days. All studies were conducted in the laboratory with a natural light/dark schedule at room temperature. During and after finishing experiments 1 and 2 (described below), snout-vent length (SVL) and head width of larvae were directly measured by calipers to the nearest 0.05 mm.

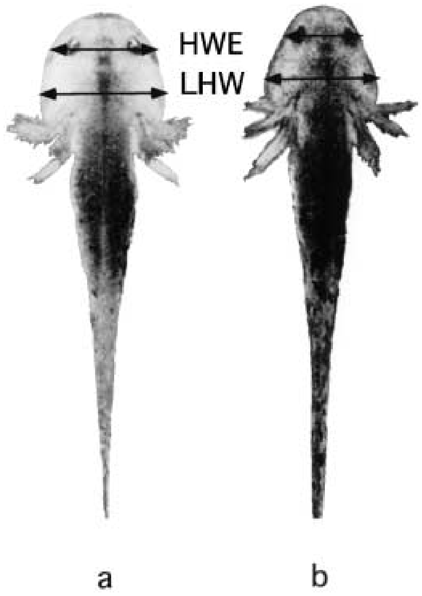

Fig. 1 External views of the broad-headed “cannibal” (**a**) and typical (**b**) morph of *Hynobius retardatus*. The “cannibal” morph is completely different from the typical morph with respect to the size of the mouth and, thus, shape of the head. To characterize the shape of the head quantitatively, we used the proportion of head width at the level of the eyes (HWE) to the largest head width (LHW). HWE/LHW≥0.9 defined a broad-headed “cannibal” morph; HWE/LHW≤0.86 was a typical morph. Larvae with 0.86<HWE/LHW<0.9 were considered as intermediate morphs

Previously, we described the broad-headed “cannibal” morph as deviating considerably from typicals in the proportion of head width to SVL (Wakahara 1995). The “cannibal” morphs were completely different from typicals with respect to the size of mouth, and thus head shape (Fig. 1). To characterize the shape of the head quantitatively, we used a proportion of head width at the level of the eyes (HWE) to the largest head width (LHW), tentatively defining a larva with HWE/LHW≥0.9 as a broad-headed “cannibal” morph and one with HWE/LHW≤0.86 as a typical morph (Fig. 1). Larvae with 0.86<HWE/LHW<0.9 were considered intermediate morphs.

Experimental design

Experiment 1

To examine whether (1) the broad-headed “cannibal” morph is induced without cannibalism and (2) the broad-headed “cannibal” morph in the absence of cannibalism achieves a larger body size than typicals, 15 randomly selected larvae which had been newly hatched from two different egg clutches collected at different sites (mixed-siblings; Fig. 2a) or developed from the same egg clutch (siblings; Fig. 2b) were reared separately in 15 cells (3×3×10 cm) partitioned with plastic mesh (mesh size 3 mm) in medium-sized plastic tanks (22×15×12.5 cm).

Three sets of the mixed-siblings (Bankei 1-Nopporo, Erimo-Maruyama, Okusawa 1-Bankei 2), and two sets of the siblings (Bankei 1 and Bankei 2) were prepared. Larvae were fed live *Tubifex* every 3 days. SVL, LHW, and HWE were measured at the end of the experiment.

Experiment 2

To identify possible factors which might influence the occurrence of the broad-headed “cannibal” morph and/or the frequency of cannibalism, three different factors – the density of larvae, levels of food supply, and kinship among conspecifics – were analyzed in this experiment. Three different larval densities (low, medium, and high) and two different levels of food supply (low and high)

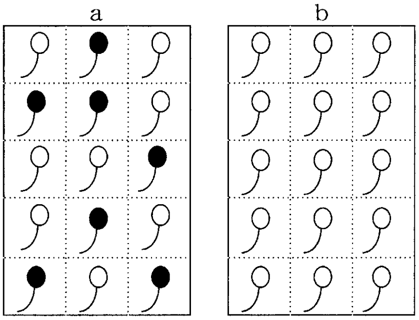

Fig. 2 Design of experiment 1. Fifteen randomly selected larvae developed from two different egg clutches collected at different sites (mixed-siblings: 8 larvae from one egg clutch and 7 from the other at the same developmental stage) (**a**) or developed from the same egg clutch (siblings) (**b**) were reared separately in 15 cells (3×3×10 cm) partitioned with plastic mesh in a medium-sized tank (22×15×12.5 cm) filled with 1.6 l of dechlorinated tap water. Three set were done with the mixed-siblings (**a**), and two with the siblings (**b**)

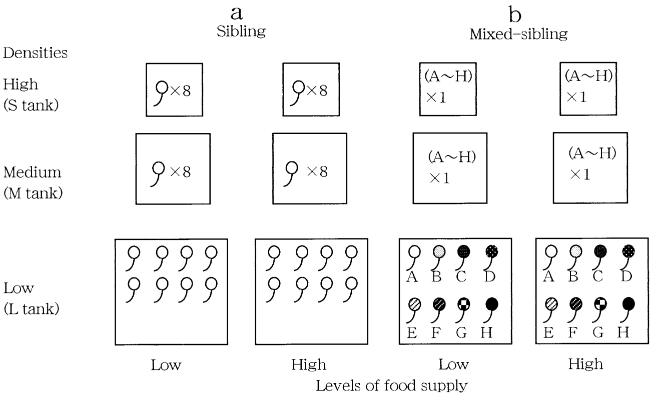

Fig. 3 Design of experiment 2. In pure-sibship experiments (**a**), 48 randomly selected larvae developed from an egg clutch were allotted to six treatments (three different larval densities×two levels of food supply), 8 larvae in each. Three types of tanks, small (*S*; 8×8×8 cm), medium (*M*; 22×15×12.5 cm), and large (*L*; 30×25×17.5 cm), were prepared to control the density of larvae. Larvae were fed live *Tubifex* every day (high food supply) or every 4 days (low food supply). These combinations of three different larval densities and two levels of food supply were applied to eight different egg clutches collected at eight different sites (Bankei 1, Bankei 2, Maruyama, Nopporo, Teine, Okusawa 1, Okusawa 2, and Erimo). In mixed-sibling experiments (**b**), 48 randomly selected larvae developed from eight different egg clutches collected from the eight different sites described above were allotted to the 48 tanks [six treatments×eight replicates (one larva from each egg clutch)]

were prepared for pure-sibship (Fig. 3a) and for mixed-sibling (Fig. 3b) experiments.

In the pure-sibship experiment, 48 randomly selected larvae, which were newly developed from an egg clutch (siblings) were allotted to six treatments (two levels of food supply×three different densities), 8 larvae each. To control the density of larvae, three types of tank, small (8×8×8 cm), medium (22×15×12.5 cm), and large (30×25×17.5 cm), were prepared,filled with 0.3, 1.6, and 3.7 l of dechlorinated tap water, respectively. Because every tank contained eight larvae, the bottomarea occupied by each individual was about 8, 41, and 94 cm^2, respectively, and the cubic capacity per individual was about 38, 188, and 469 cm^3, respectively. They were fed live *Tubifex* every day (high food supply) or every 4 days (low food supply). These six treatments were replicated using eight different egg clutches (eight siblings) collected at eight different sites, Bankei 1, Bankei 2, Maruyama, Nopporo, Teine, Okusawa 1, Okusawa 2 and Erimo.

In the mixed-sibling experiment, every tank contained eight larvae, each of which was derived from a different egg clutch, collected at Bankei 1, Bankei 2, Maruyama, Nopporo, Teine, Okusawa 1, Okusawa 2, and Erimo(Fig. 3b). Similar, to the pure-sibship experiment, six treatments (two levels of food supply×three different densities) were replicated using eight sets of the mixed-sibling combination of larvae.

Larvae were supplied with food for 1 h from 1200 to 1300 hours. Larvae were always fed with enough food to eat within 1 h, and any food remaining in their tanks was removed after the feeding period. Numbers of the broad-headed "cannibal," intermediate, and typical morphs were counted every morning. The frequency of cannibalism was estimated by counting the number of cannibals which had cannibalized conspecifics in each treatment. Since the broad-headed "cannibal" morph continuously consumes conspecifics once the morph is established (Wakahara 1995), the number of larvae in tanks decreases drastically due to continuous cannibalism. Thus, cannibals which had consumed conspecifics were removed from the tanks after the cannibalism.

Statistical analyses

In experiment 1, we used one-way ANOVA to analyze the means of SVL among the three morphotypes (broad-headed "cannibal," intermediate, and typical morphs). In experiment 2, we examined possible relationships between the occurrence rate of the broad-headed "cannibal" morph or the frequency of cannibalism and three environmental factors, larval density, level of food supply, and kinship. There were 12 treatments (3 different larval densities×2 levels of food supply×2 levels of kinship). Because all experiments were replicated eight times within a treatment, we observed a total of 768 larvae using 96 different tanks. The occurrence rate of the broad-headed "cannibal" morph was expressed as the frequency of "cannibal" morphs per individual. The frequency of cannibalisms was expressed as the frequency of cannibals observed per individual. Because all data were expressed as proportions, we conducted arcsine transformation on the data to improve statistical power. We used multiway analysis of variance (three-way ANOVA) to analyze the occurrence rate of the broad-headed "cannibal" morph and the frequency of cannibalism.

Results

The broad-headed "cannibal" morph is induced without cannibalism

Table 1 shows the number of broad-headed "cannibal" (HWE/LHW$\geq$0.9), intermediate (0.86<HWE/LHW<0.9), and typical (HWE/LHW$\leq$0.86) morphs (cf. Fig. 1) which were induced without cannibalism in five tanks of experiment 1. The broad-headed "cannibal" morph was induced without cannibalism in almost all tanks regardless of their combination, either sibling or mixed-sibling groups. Thus the broad-headed "cannibal" and intermediate morphs do not arise as a result of cannibalism, but can be induced without actual cannibalism.

When the body size of the larvae of the different morphs was compared at the end of the experiment, there were significant differences among the SVL means of the broad-headed "cannibal," intermediate, and typical morphs (one-way ANOVA: $F_{2,71}$=22.778, P<0.0001). The broad-headed "cannibal" and intermediate morphs

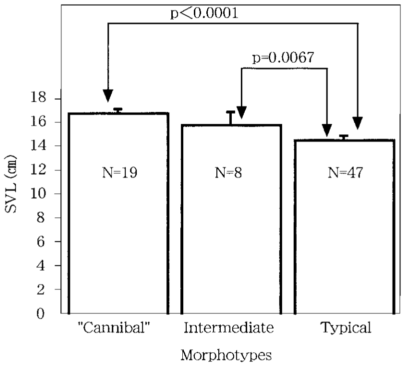

Fig. 4 Comparison of the snout-vent length (SVL; mean±SE) among broad-headed “cannibal,” intermediate, and typical morphs in experiment 1 on the 14th day rearing (see Table 1). There are significant differences among the means of broad-headed “cannibal,” intermediate, and typical morphs (one-way ANOVA: $F_{2,71}$=22.778, P<0.0001). *Arrows* indicate statistically significant differences between the means (Fisher’s PSLD)

had a tendency to grow larger in SVL than typicals even without cannibalism [Fisher’s PSLD: broad-headed “cannibal” morph (n=19) vs typicals (n=47), P<0.0001; intermediate morph (n=8) vs typicals (n=48), P=0.0067; Fig. 4].

Frequency of cannibalism

This study confirmed the results of preliminary studies that even typical morphs are capable of cannibalizing conspecifics. However, the “cannibalistic larvae with typical morph” ate only one larva and did not show continuous cannibalism, while the broad-headed “cannibal” morph continuously consumed conspecifics once the morph was established (Wakahara 1995). Thus, cannibals which had consumed conspecifics were removed from the tanks just after the cannibalism event.

Over the 14 days of experiment 2, the frequency of cannibalisms was significantly larger at highthan at low larval densities ($F_{2,84}$=4.651, P=0.0121; Fig. 5a, Table 2), at low than at high levels of food supply ($F_{1,84}$=4.676, P=0.0334; Fig. 5b, Table 2), and in mixed-sibling groups than in the pure sibships ($F_{1,84}$=4.921, P=0.0292; Fig. 5c, Table 2). In the three different larval density treatments, the frequency of cannibalisms was significantly larger at high or medium density than at low density (Fisher’s PSLD: high density vs low density, P=0.0038; medium density vs low density, P=0.0433). The ANOVA for larval density, level of food supply, and kinship among larvae revealed significant effects of these treatments on cannibalism when examined singly (Table 2). However the interactions of density×food×kinship ($F_{2,84}$=0.442, P=0.6442), density×food ($F_{2,84}$=1.206, P=0.3047), density×kinship ($F_{2,84}$=1.204, P=0.3051), and food×kinship ($F_{1,84}$=0.047, P=0.8292), were not significant (Table 2).

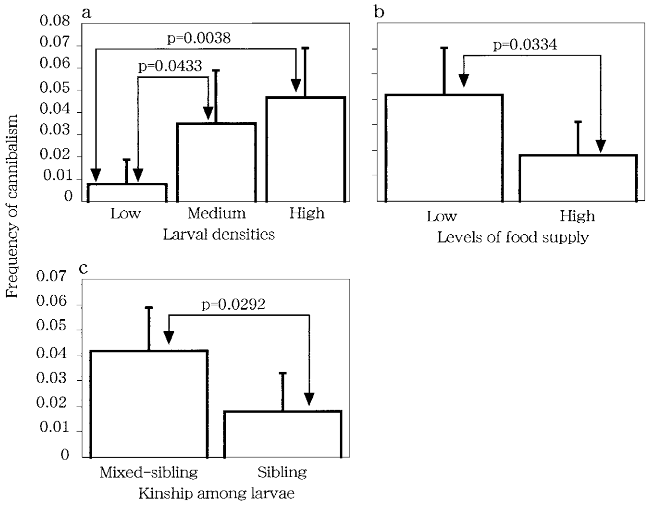

Fig. 5 The frequency (mean±SE) of cannibalism per individual analyzed for the effects of larval density (**a**), level of food supply (**b**), and kinship among larvae (**c**). *Arrows* indicate statistically significant differences between the means (Fisher’s PSLD)

Occurrence of the broad-headed “cannibal” morph

Over the 14 days of experiment 2, the broad-headed “cannibal” morphs were more frequently induced in the mixed-sibling groups of higher conspecific density than in those of medium density, and in the pure-sibling groups of higher density, as indicated by the significant density×kinship interaction ($F_{2,84}$=7.177, P=0.0013; Fig. 6, Table 3). The ANOVA of larval density, level of food supply, and kinship among larvae revealed single and/or interactive effects of these treatments on the occurrence of the “cannibal” morph (Table 3). Interactions were not significant: density×food×kinship ($F_{2,84}$=0.089, P=0.9153), density×food ($F_{2,84}$=0.266, P=0.7672), and food×kinship ($F_{1,84}$=0.089, P=0.7667), (see Table 3). In

Table 1 Number of larvae which showed the “cannibal,” intermediate, and typical morph type in three combinations of mixed-siblings and two of siblings in experiment 1

	Combination	Morphotype		
		“Cannibal”	Intermediate	Typical
Mixed-siblings	Bankei 1-Nopporo	8	3	4
	Bankei 2-Maruyama	0	1	14
	Erimo-Okusawa 1[a]	6	1	7
Siblings	Bankei 1	1	0	14
	Bankei 2	3	3	9

[a] One larva died in this combination

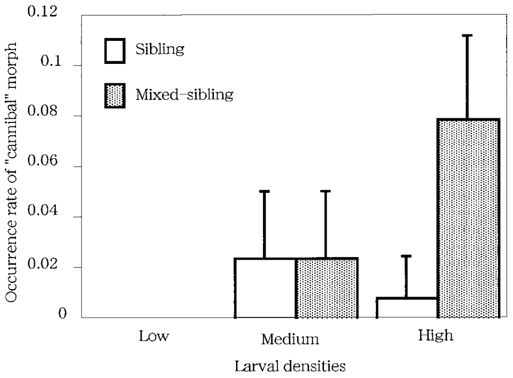

Fig. 6 The occurrence rate of the broad-headed "cannibal" morph per individual (mean±SE), analyzed for the interactive effects of larval density and kinship among larvae. The "cannibal" morphs were more frequently induced in the mixed-sibling groups at higher density than in those of medium density and in the pure-sibling groups of higher density

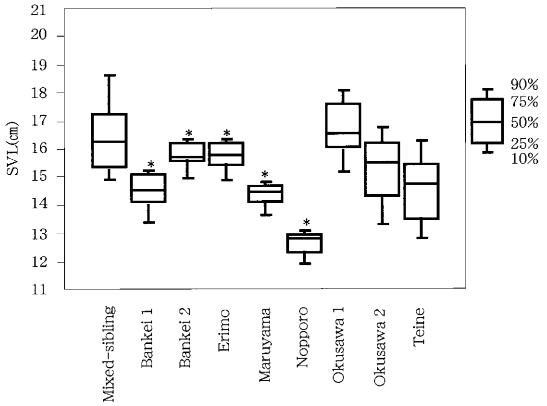

Fig. 7 Variation in SVL of sibling larvae (Bankei 1, Bankei 2, Maruyama, Nopporo, Okusawa 1, Okusawa 2, Teine: 8 larvae each; Erimo: 7 larvae) and 62 mixed-sibling larvae. These were derived from medium-density tanks with a high food supply in experiment 2 (see Fig. 3). *Asterisks* indicate a significantly smaller variation in SVL in siblings of five clutches (Bankei 1, Bankei 2, Erimo, Maruyama, and Nopporo) than among the mixed-siblings. For siblings of the other three clutches (Okusawa 1, Okusawa 2, and Teine), variation in SVL was as large as in the mixed-siblings

contrast to the results of actual cannibalism described above (Fig. 5b, Table 2), however, there were no significant effects of the level of food supply on the occurrence of the "cannibal" morph ($F_{1,84}$=0.797, P=0.3744; Table 3).

Almost all larvae in the tanks of the mixed-sibling groups did not show the broad-headed "cannibal" morphs even at the high density of conspecifics, but a small number did. Only 10 larvae of 128 (8 mixed-sibling larvae×8 tanks×2 levels of food supply) at high density and 3 larvae of 128 at medium density became the "cannibal" morph. Unfortunately, however, we could not determine from which clutch(es) the "cannibal" morphs were derived, because the larvae in the mixed-sibling groups were not marked individually in this experiment.

Variation of body size of larvae in sibling and in mixed-sibling groups

Although we intended to use similar-sized larvae in these experiments, larvae with some variation in body size might have been used in experiments 1 and 2 because precise measurements of the SVL of larvae were very difficult to make, due to their small size and ease of injury. Thus, to examine whether variation in the body size of larvae affected the occurrence of the broad-headed "cannibal" morph, we analyzed SVL data of larvae with-

Table 2 ANOVA table for the effects of larval density, level of food supply, and kinship among larvac on the frequency of cannibalism. Data were arcsine transformed before analysis

Source of variance	*df*	MS	*F*	*P*
Larval density	2	0.027	4.651	0.0121
Level of food supply	1	0.028	4.676	0.0334
Kinship among larvae	1	0.029	4.921	0.0292
Larval density×level of food supply	2	0.007	1.206	0.3047
Larval density×kinship among larvae	2	0.007	1.204	0.3051
Level of food supply×kinship among larvae	1	2.766E-4	0.047	0.8292
Larval density×level of food supply×kinship among larvae	2	0.003	0.442	0.6442
Residual	84	0.006		

Table 3 ANOVA table for the effects of larval density, level of food supply, and kinship among larvae on the occurence rate of the broad-headed "cannibal" morph. Data were arcsine transformed before analysis

Source of variance	*df*	MS	*F*	*P*
Larval density	2	0.044	11.253	<0.0001
Level of food supply	1	0.003	0.797	0.3744
Kinship among larvae	1	0.028	7.177	0.0089
Larval density×level of food supply	2	0.001	0.266	0.7672
Larval density×kinship among larvae	2	0.028	7.177	0.0013
Level of food supply×kinship among larvae	1	3.499E-4	0.089	0.7667
Larval density×level of food supply×kinship among larvae	2	3.499E–4	0.089	0.9153
Residual	84	0.004		

out cannibalism at the end of the experiment. All larvae analyzed here were derived from experiment 2: 63 sibling larvae (8 larvae each from Bankei 1, Bankei 2, Nopporo, Okusawa 1, Okusawa 2, Teine, and Maruyama, and 7 larvae from Erimo) in the medium-density and high-food-supply treatment, and 62 mixed-sibling larvae of the same treatment (see Fig. 3). There was significantly larger variation in SVL in the mixed-siblings than in the siblings of five clutches (Bankei 1, Bankei 2, Nopporo, Maruyama, and Erimo; *F*-test: Bankei 1 vs mixed, $F_{7,60}=5.082$, $P=0.0151$; Bankei 2 vs mixed, $F_{7,60}=7.912$, $P=0.039$; Nopporo vs mixed, $F_{7,60}=10.044$, $P=0.0018$; Maruyama vs mixed, $F_{7,60}=11.359$, $P=0.0012$; Erimo vs mixed, $F_{6,60}=6.552$, $P=0.0122$) (Fig. 7). In contrast, there was no significant evidence of variation of SVL between the mixed-siblings and three other clutches (Okusawa 1, Okusawa 2, and Teine; *F*-test: Okusawa 1 vs mixed, $F_{7,60}=2.033$, $P=0.2285$; Okusawa 2 vs mixed, $F_{7,60}=1.328$, $P=0.6248$; Teine vs mixed, $F_{7,60}=1.208$, $p=0.7443$) (Fig. 7).

We observed no broad-headed “cannibal” morphs in tanks of Okusawa 1, Okusawa 2, and Teine, which showed considerable variation in SVL, similar to the mixed-sibling group. In contrast, one broad-headed “cannibal” morph was observed in each tank of Bankei 1, Bankei 2, Nopporo, Maruyama, and Erimo, even though they showed significantly smaller variation in SVL than the mixed-sibling group. These results suggest that the occurrence of the broad-headed “cannibal” morph was independent of the variation in body size.

Discussion

Relationship of cannibalism and environmental factors

Our studies demonstrate that the frequency of cannibalism (number of cannibals/individual) is significantly higher at highthan at low density, at low than at high food supply, and among mixed-sibling than in pure sibling groups (Fig. 5, Table 2). The results reported here support previous studies in *H. retardatus* by Wakahara (1995, 1997). These three factors affecting cannibalism are similar to those reported in *A. tigrinum nebulosum* (Collins and Cheek 1983; Pfennig and Collins 1993). The interactions of density×food×kinship, density×food, density×kinship, and food×kinship on cannibalism were not significant (Table 2), suggesting that the three factors affected actual cannibalism independently of each other in this species.

Because *H. retardatus* spawns in early spring in ponds partially covered with snow and ice (Sato 1989; Iwasaki and Wakahara 1999), the newly hatched, carnivorous larvae are probably exposed habitually to a shortage of prey except for a few, overwintered aquatic insects and newly hatched tadpoles of *Rana pirica*, which breeds in similar habitats to *H. retardatus* (Ohdachi 1994), but a little later than *H. retardatus*. If no prey are available, it may be adaptive for larvae to consider conspecifics as a good prey item and consume them. Thus, larvae will be selected to consume conspecifics at higher larval densities and in lower-food-supply environments. However, indifferent cannibalism may diminish the component of their inclusive fitness by killing relatives (Hamilton 1964; Waldman 1988; Walls and Roudebush 1991). Our results showing that cannibalism was much more suppressed among siblings than with mixed siblings (Fig. 5), independent of the other two factors (Table 2), are consistent with Hamilton’ (1964) theory of inclusive fitness.

Factors inducing the broad-headed “cannibal” morph

In experiment 1, we found that the broad-headed “cannibal” morphs were induced without actual cannibalism (Table 1). This result indicates that cannibalism itself is not necessary to induce the broad-headed “cannibal” morphs in *H. retardatus*, as reported previously (Nishihara 1996). Because the “cannibal” morph, once established, tends to grow larger than the typicals without actual cannibalism (Fig. 4), there might be some genetic differences between those embryos that eventually express the “cannibal” morph and those that express the typical-morph features.

Experiment 2 demonstrated that the broad-headed “cannibal” morph was affected by interactive effects of density×kinship but not by the levels of food supply (Table 3). The broad-headed “cannibal” morphs were more frequently induced in the mixed-sibling groups at a higher density of conspecifics than in mixed-sibling groups of medium density and in the pure-sibling groups at higher density (Fig. 6, Table 3). Initial expression of the broad-headed “cannibal” morph was observed within 7 days after hatching in 15 out of 18 larvae in experiment 2 (data not shown). These results suggest that newly hatched larvae (stage 41 according to Iwasawa and Yamashita 1991) are able to modify their morphology or select their morphotype before feeding (stage 44) by receiving some specific cues from their kinships and density. Hoffman and Pfennig (1999) have similarly shown that younger larvae of *A. tigrinum* were more likely than older larvae to develop into cannibals because the former appear to be more sensitive to cues that induce cannibals. Although increased variation in larval size distribution also appears to stimulate development of cannibal morphs in *A. tigrinum* (Maret and Collins 1994), the occurrence of the broad-headed “cannibal” morph in *H. retardatus* is independent of the variation in body size among larvae within a tank (Fig. 7), suggesting, again, that there might be some genetic differences between embryos that will express the “cannibal” morph and those that do not. Our next step will be to elucidate the possible genetic difference in different populations of *H. retardatus*.

Adaptive function of the broad-headed “cannibal” morph

The broad-headed “cannibal” morphs may have some functional advantages in feeding and handling larger conspecifics. Large-headed cannibals in *H. retardatus* have been reported to be at an advantage for eating conspecifics and consuming tadpoles of *R. pirica* (Ohdachi 1994). Similarly, cannibal morphs of *A. tigrinum* exceed typicals in feeding and handling larger larvae (Hoffman and Pfennig 1999; Stephen et al. 1992).

It seems reasonable to assume that larvae which can modify or select their morphology and express the broad-headed "cannibal" morph according to density have an adaptive advantage for capturing and consuming conspecificswhen the future prey density is rich. Therefore, the broad-headed "cannibal" morphs may be induced frequently at higher larval densitiesy (Fig. 6, Table 3).

There are two critical issues concerning the ecological significance of the broad-headed "cannibal" morph in this species. The first relates to why the broad-headed "cannibal" morph occurred so frequently in mixed-sibling groups at higher density, and why it was suppressed among siblings even at high density (Fig. 6, Table 3). The broad-headed "cannibal" morph continuously consumes conspecifics once it is established (Wakahara 1995), though cannibalism was basically suppressed among siblings (Wakahara 1997). Because the broad-headed "cannibal" morphs do eat siblings (Wakahara 1995), they may not accurately recognize kinship or they may eat siblings to avoid starvation even though they canrecognize discriminate kin. Even though the broad-headed "cannibal" morph is an adaptive morphotype which can consume larger conspecifics at high larval densities, because it may injure or eat relatives, the morphotype must be suppressed among relatives.

The second issue is why all members in tanks of the mixed-sibling groups in experiment 2 did not express the broad-headed morph, which is better than typicals at capturing and eating larger larvae, even though there was no risk of eating kin in the tanks of mixed-sibling groups (cf. Fig. 3b). In general, cannibal morphs may be favored when the benefits of cannibalism, such as faster growth and higher survivorship (Fox 1975; Polis 1981; Crump 1990), surpass its costs (Pfennig et al. 1998). Indeed, the broad-headed "cannibal" morph that continuously consumes conspecifics has been reported to show faster growth during the larval period and to achieve faster metamorphosis in *H. retardatus* (Wakahara 1995). Given these advantages and the fact that in the mixed-sibling tanks there were no associated costs of consuming relatives, all larvae in the mixed-sibling tanks might be expected to show the broad-headed "cannibal" morph. But they did not.. The explanation may be selection in the natural habitat for polymorphism in the ability to express the "cannibal" morph, arising fromfrequency-dependent selection on the parent's reproductive success and the inclusive fitness of the "cannibal" morph.

Acknowledgements We thank Drs Kinya Nishimura, Faculty of Fisheries, and Satoru Araki, Graduate School of Environmental Earth Science, Hokkaido University, for helpful discussion. All animal manipulations reported here were carried out in accordance with the legal and ethical standards of the Japanese authorities.

References

Arai T, Wakahara M (1993) Hemoglobin transition from larval to adulttypes in normally metamorphosing, metamorphosed and metamorphosis-arrested *Hynobius retardatus*. Zool Sci 10:637–644

Collins JP, Cheek JE (1983) Effect of food and density on development of typical and cannibalistic salamander larvae in *Ambystoma tigrinum nebulosum.* Am Zool 23:77–84

Crump ML (1986) Cannibalism by younger tadpoles: another hazard of metamorphosis. Copeia 1986:1007–1009

Crump ML (1990) Possible enhancement of growth in tadpoles through cannibalism. Copeia 1990:560–564

Elgar MA, Crespi BJ (1992) Cannibalism: ecology and evolution among diverse taxa. Oxford University Press, Oxford

Fox LR (1975) Cannibalism in natural populations. Annu Rev Ecol Syst 6:87–106

Hamilton WD (1964) The genetical evolution of social behavior. I and II. J Theor Biol 7:1–52

Hoffman EA, Pfennig DW (1999) Proximate causes of cannibalistic polyphenism in larval tiger salamanders. Ecology 80:1076–1080

Iwasaki F, Wakahara M (1999) Adaptable larval life histories in different populations of the salamander, *Hynobius retardatus*, living in various habitats. Zool Sci 16:667–674

Iwasawa H, Yamashita K (1991) Normal stages of development of a hynobiid salamander, *Hynobius nigrescens* Stejneger. Jpn J Herpetol 14:39–62

Lannoo MJ, Bachmann MD (1984) Aspects of cannibalistic morphs in a population of *Ambystoma t. tigrinum* larvae. Am Mid Nat 112:103–109

Maret TJ, Collins JP (1994) Individual responses to population size structure: the role of size variation in controlling expression of a trophic polyphenism. Oecologia 100:279–285

Meffe GK, Crump ML (1987) Possible growth and reproductive benefits of cannibalism in the mosquitofish. Am Nat 129:203–212

Nishihara A (1996) Effects of density on growth of head size in larvae of the salamander *Hynobius retardatus.* Copeia 1996:478–483

Ohdachi S (1994) Growth, metamorphosis, and gape-limited cannibalism and predation on tadpoles in larvae of salamanders, *Hynobius retardatus*. Zool Sci 11:127–131

Pfennig DW, Collins JP (1993) Kinship affects morphogenesis in cannibalistic salamander. Nature 362:836–838

Pfennig DW, Frankino WA (1997) Kin-mediated morphogenesis in facultatively cannibalistic tadpoles. Evolution 51:1993–1999

Pfennig DW, Reeve HK, Sherman PW (1993) Kin recognition and cannibalism in spadefoot toad tadpoles. Anim Behav 46:87–94

Pfennig DW, Ho SG, Hoffman EA (1998) Pathogen transmission as a selective force against cannibalism. Anim Behav 55: 1255–1261

Polis GA (1981) The evolution and dynamics of intraspecific predation. Annu Rev Ecol Syst 12:225–251

Sato T (1989) Breeding environment and spawning of a salamander, *Hynobius retardatus*, at the foot of Hidaka Mountains, Hokkaido. In: Matsui M, Hikida T, Goris RC (eds) Current herpetology in East Asia. Herpetology Society of Japan, Kyoto, pp 292–304

Stephen MR, George VL, James PC (1992) Performance consequences of a trophic polymorphism: feeding behavior in typical and cannibal phenotypes of *Ambystoma tigrinum*. Copeia 1992:672–679

Wakahara M (1995) Cannibalism and resulting dimorphism in larvae of a salamander *Hynobius retardatus*, inhabited in Hokkaido, Japan. Zool Sci 12:467–473

Wakahara M (1997) Kin recognition among intact and blinded, mixed-sibling larvae of a cannibalistic salamander *Hynobius retardatus*. Zool Sci 14:893–899

Waldman B (1988) The ecology of kin recognition. Annu Rev Ecol Syst 19:543–571

Walls SC, Roudebush RD (1991) Reduced aggression toward siblings as evidence of kin recognition in cannibalistic salamander. Am Nat 138:1027–1038