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Kin Recognition among Intact and Blinded, Mixed-Sibling Larvae of a Cannibalistic Salamander *Hynobius retardatus*

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ABSTRACT—Cannibalisms among larvae of a salamander *Hynobius retardatus* were affected by level of food supply, their density, body size and kinship. Kin recognition during cannibalisms among mixed-sibling larvae was examined using larvae with similar body size (within 10% differences in total body length at hatching stage) developed from different egg clutches. In order to distinguish larvae developed from an egg clutch from ones developed from different egg clutches, individual marking system by eye ball-removal operation was conducted. The eye ball-removal itself had no effect on the cannibalism, either to consume or to be comsumed. It was found that the larvae can recognize kinship without sence of sight and preferentially consume non-kins or avoid killing siblings, when larvae hatched from different egg clutches were mixed. The cannibalism, however, was either induced or not among larvae hatched from an egg clutch. The destiny of larvae from an egg clutch whether they became "cannibalistic" or "non-cannibalistic" was determined in every egg clutch by observing the cannibalism in larvae hatched from each egg clutch, respectively, during the first 10 days after hatching. Thus, it was possible to distinguish "cannibalistic" clutches which contained cannibalistic larvae from "non-cannibalistic" clutches which never showed the cannibalism. Even larvae of the "non-cannibalistic" clutch, however, had an ability to eat another individual when mixed with larvae hatched from different egg clutches.

INTRODUCTION

Many animals are known to modify their behaviour towards kin in a fashion consistent with the inclusive fitness theory (Hamilton, 1964). In accordance with the theory, cannibalisms are induced significantly more frequently in mixed-blood groups than the pure sibship groups in cannibalistic salamanders (Pfennig and Collins, 1993; Pfennig *et al.*, 1994; Sadler and Elgar, 1994). A salamander *Hynobius retardatus* living in Hokkaido, Japan, which had been reported to show neotenic reproduction (Sasaki, 1924; Wakahara, 1996), shows a cannibalism during larval stages (Ohdachi, 1994; Wakahara, 1995; Nishihara, 1996) and a resultant dimorphism, "typical" non-cannibals and "broad-headed" cannibals which facilitate the ingestion of conspecifics (Wakahara, 1995). In the previous paper, it was reported that the frequency of cannibalism in siblings was significantly lower than in the mixed-sibling groups (Wakahara, 1995). This result suggests that the larvae of *Hynobius retardatus* recognize kinship like the Arizona tiger salamander (Pfennig and Collins, 1993) and that even the cannibalistic morph avoids killing siblings when given a choice between siblings, cousins and non-kin, and preferentially eats unrelated larvae when presented with cousins and unrelated individuals (Sadler and Elgar, 1994).

Because individual marking has not been introduced in mixed-sibling experiments to date, it is impossible to make sure directly whether the cannibals certainly recognize kinship and preferentially consume non-kins or avoid killing siblings. In order to distinguish the larvae developed from a clucth from another in mixed-sibling experiments, lateral eyes were surgically removed. This report shows that larvae can recognize kinship and avoid consuming siblings without the sence of sight.

MATERIALS AND METHODS

Animals

Fertilized eggs of *Hynobius retardatus* were collected from several ponds or small streams in the vicinity of Sapporo, Japan; Bankei (B), Nopporo (N), Okusawa (O) and Toyoha (T) during the breeding seasons (from early April to late May) in1995, 1996 and1997. Each egg clutch was placed separately in different small plastic tanks (22 × 15 × 13 cm) filled with 2 l of dechlorinated tap water supplemented with antibiotics (100 IU/ml penicillin G and 100 μg/ml streptomycin) at 4°C until use. Before experiments, prehatched embryos (stage 20-25 according to the normal table for *Hynobius nigrescens*, a closely related species to *H. retardatus*) were reared at a room temperature until they hatched at stages 40-41. After total body lengths of all the larvae hatched from their egg capsules were directly measured by slide calipers to the nearest 0.05 mm, they were used for experiments described below. Hatched larvae were fed with live freshwater oligocheate (*Tubifex*) or with commercially available frog feed pellets

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(Oriental Kobo Co., No. 2 for frogs).

Experimental design

Four experiments were conducted to evaluate possible ecological significance of cannibalism among larvae, relationship between the kinship and the frequency of cannibalism, and to know possible cues for kinship recognition during the cannibalism. All experiments were done during the summer seasons (June - August) from 1995 to 1997 at room temperatures (18-23°C). Experiment I was done in 1995, Experiments II and III were repeatedly performed in 1996 and 1997, and Experiment IV was done in 1996.

Experiment I. In order to know possible ecological significance of the cannibalism among larvae, body size at and time until metamorphosis were compared between cannibals and non-cannibals. Fourty larvae at stages 40-41 developed from different egg clutches were reared in 8 large plastic tanks (35 × 30 × 18 cm). Some larvae which had eaten conspecifics at once continued to eat them and became to show a "broad-headed", cannibalistic morph, but others did not consume conspecifics at all and showed a "typical", non-cannibalistic morph (Wakahara, 1995). Duration until their completion of metamorphosis (days after hatching) was recorded in the cannibals and non-cananibals, respectively. Wet body weight and snout-vent length (SVL) at their completion of metamorphosis were also measured to the nearest 0.001 gm and 0.05 mm for each individual, respectively.

Experiment II. Because body size of larvae developed from different egg clutches or even from one egg clutch were very diverse (see Fig. 2), and the cannibalism depended on the size of larvae (Wakahara, 1995), the size of experimental larvae must be strictly controlled when comparing frequency of cannibalisms between siblings and mixed-sibling groups. Degrees of body size-differences among larvae reared in a tank were expressed by "percent difference in total body length" at the hatching stages (stages 40-41), which was calculated as follows: (maximal total length—minimal total length of larvae in one tank)/average length. Three size-difference groups were prepared in sibling as well as in mixed-sibling groups as follows; within 10%, between 10 to 20%, and over 20% differences in total body length. Thirty larvae developed from one egg clutch (siblings) or developed from two egg clutches (mixed-siblings) were grown in each small plastic tank (22 × 15 × 13 cm). Eighteen tanks of siblings and 24 tanks of mixed-sibling groups were prepared. They were fed once a day with a medium level of food supply (see Wakahara, 1995). Numbers of victims by cannibalisms and dead larvae were daily recorded respectively, for 30 days.

Experiment III. From the results of Experiment II and several preliminary experiments, it was concluded that each group developed from the same clutch could be categorized into either "cannibalistic" group in which cannibals became to appear, or "non-cannibalistic" group which never showed cannibalisms (see Fig. 4). In order to know whether larvae of the "non-cannibalistic" group have an ability to eat conspecifics or not, the larvae which had been identified as "non-cannibalistic" were mixed with other "non-cannibalistic" larvae developed from different egg clutches. Body size-differences of larvae reared in one tank were controlled within 12.5%, when the experiment was done (10 days after hatching).

Experiment IV. In order to know what cues the larvae use to recognize sibship and avoid killing close relatives, and also to distinguish larvae from one clutch and ones from another clutch in a tank, eye balls were removed from larvae of one clutch and then mixed with intact larvae of another clutch. After anesthesia by immersing the larvae in MS-222 (Sandoz, 1/2000 in Steinberg's solution), both eye balls were removed using a pair of Wickel's scissors. After leaving them in Steinberg's solution for 24 hr for wound healing, they were mixed with intact larvae of either the same clutch or another clutch. Body size-differences of larvae were controlled within 10% at hatching stages. Each tank contained at first 30-40 larvae, a half of which were subjected to the eye removal operation. For two-sibship experiments, "cannibalistic" groups (such as O5 in Fig. 4) and "non-cannibalistic" groups (such as B1 in Fig. 4), both of which had been determined by rearing sibling larvae for 10 days after hatching, were mixed. Twenty tanks were prepared, half of which (tank no. 11-20) contained "non-cannibalistic" intact and "canniblistic" blinded larvae. Another half (tank no. 21-30) contained "cannibalistic" intact and "non-cannibalistic" blinded larvae. For one-sibship experiments, "non-cannibalistic" groups were chosen. A half of larvae were blinded, and then mixed with intact larvae of the same group (tank no. 31-35). In order to know which larvae, intact or blinded of the same clutch, were preferentially consumed by cannibalisms, 2 cannibals from different clutches were introduced to the tanks (tank no. 36-40). The number of victims by cannibalisms and dead larvae of either intact or blinded were daily recorded respectively, for 25 days.

RESULTS

Cannibals grow faster than non-cannibals

When 40 larvae of *Hynobius retardatus* developed from different egg clutches were reared in one large tank, some larvae became to consume the conspecifics, but others did not eat the conspecifics at all. The cannibalism was frequently observed during early developmental stages until 25 days after hatching, but not observed thereafter. The larvae which ate the conspecifics at once continued to consume them. The cannibals developed to "broad-headed", but the non-cannibals to "typical" morphs (Wakahara, 1995). The final number of cannibals in one tank was relatively small (1 to 4 per tank). When 320 larvae were reared in 8 tanks, 24 cannibals and 107 non-cannibals completed their metamorphosis. The lost (189 larvae) were consumed by cannibalisms or accidentally died. The broad-headed, cannibals metamorphosed significantly earlier than the typical, non-cannibals (Fig. 1). The cannibals grew faster and metamorphosed earlier (in average,

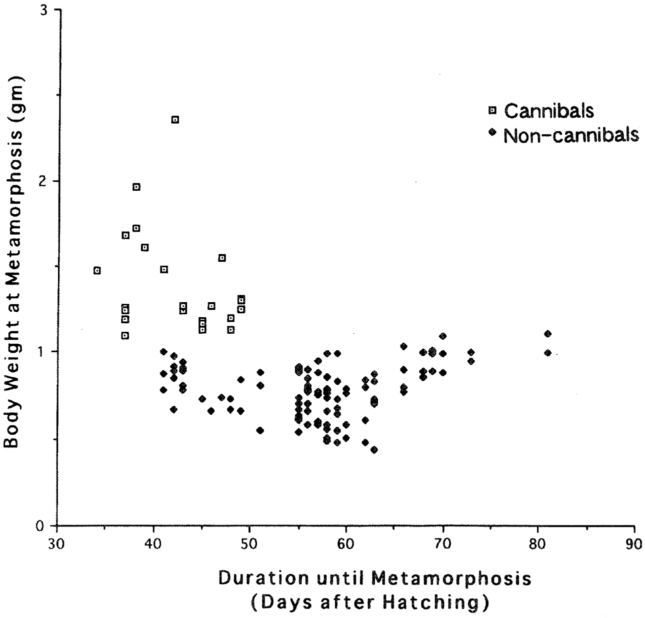

Fig. 1. Body weight at and time until metamorphosis of cannibals and non-cannibals in *Hynobius retardatus*. The wet weight was measured at their completion of metamorphosis.

42.2 days after hatching; n = 24) than the non-cannibals (60.2 days; n = 107, $p < 0.05$; Mann-Whitney *U*-test). Mean body weight at the metamorphosis was 1.35 gm in the cannibals and 0.85 gm in the non-cannibals ($p < 0.001$; Mann-Whitney *U*-test). The average body (snout-vent) length of the cannibals at the completion of metamorphosis was also significantly larger than the non-cannibals.

Cannibalism is suppressed among siblings

Total body lengths of larvae at hatching stages were very diverse from an egg clutch to others (Fig. 2). The largest was 21.2 mm in average total length (B-12), while the smallest was 15.7 mm (B-02), so far as examined. Ranges of the body size (maximal—minimal) in siblings were also very diverse: some egg clutches produced larvae with relatively narrower ranges (e.g., S-06, S-01, O-14, S-05), but the others produced larvae with wider ranges (eg., B-12, T-04, O-11). Thus, it was possible to arrange experimental larvae with either similar or different body size in both sibling and mixed-sibling groups. In order to assess possible correlation among cannibalisms, kinship and body size of larvae reared in a tank, degrees of cannibalisms were monitored in three size-difference groups; within 10%, between 10 to 20%, and over 20% differences in total body length at the hatching stages.

Figure 3 shows per cent reduction of larvae by cannibalisms for 30 days among larvae hatched from one egg clutch (siblings, open columns), and among from two different egg clutches (mixed-siblings, hatched columns). In similar body size groups (within 10% difference in total body length), cannibalisms were basically suppressed in sibling groups; no cannibalisms were observed at all in 6 of 7 tanks. Contrary to this, moderate cannibalisms were induced in mixed-sibling groups with similar body size; cannibalisms were induced in all the tanks. The differences in the % reduction of larvae between the sibling and mixed-sibling groups were significant ($p < 0.01$ by Mann-Whitney *U*-test). Similar results were obtained in the groups of 10-20% difference in total body length; in 2 of 6 tanks in the sibling groups no cannibalisms were observed at all, whereas cannibalisms were inevitably induced in all tanks of the mixed-sibling groups. The differences in the % reduction of larvae between the sibling and

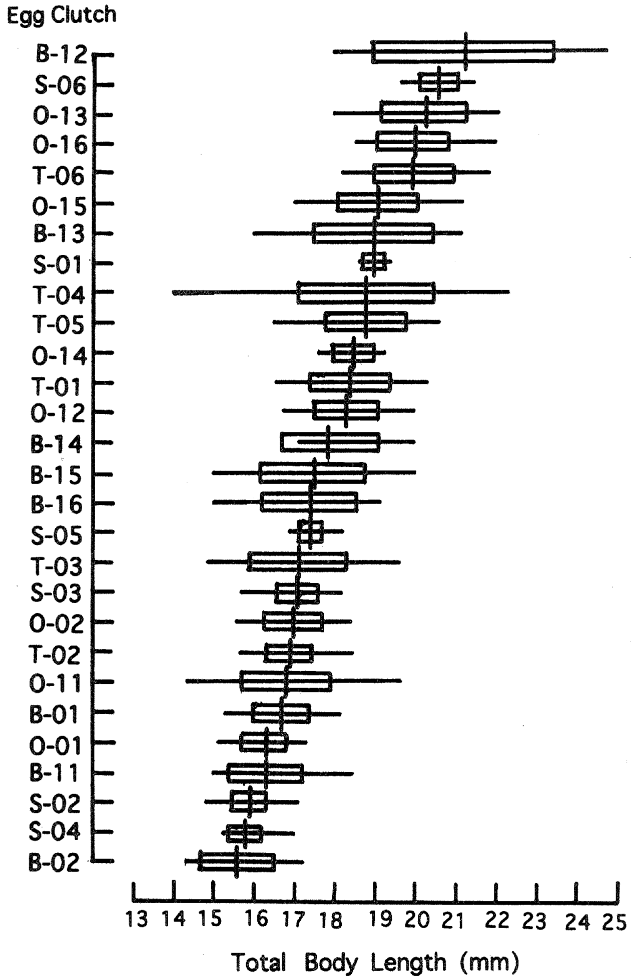

Fig. 2. Intersibling and intrasibling variations in body size of larvae hatched from 28 egg clutches collected from several breeding fields. Total body lengths were measured at hatching stages (stages 40-41). Vertical lines, average; open rectangles, ± SD; horizontal lines, ranges (minimal to maximal).

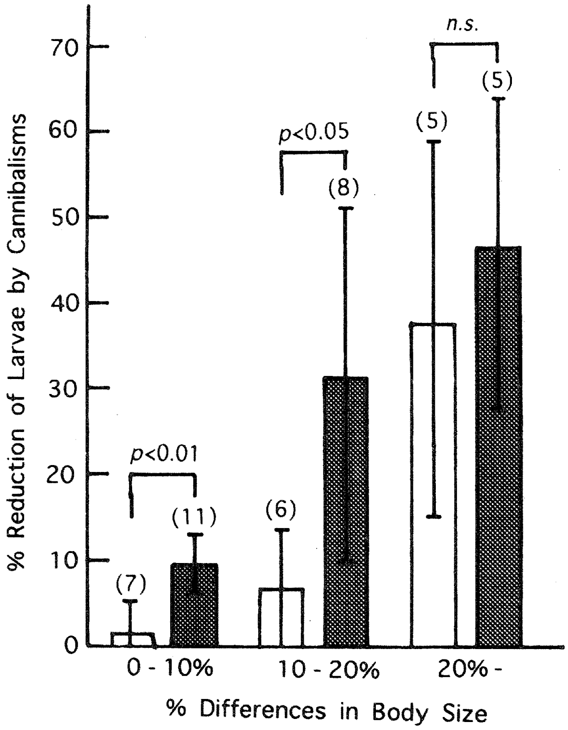

Fig. 3. Percent reduction of larvae by cannibalisms among larvae which developed from the same egg clutches (siblings, open columns) and among larvae from different egg clutches (mixed-siblings, hatched columns), in three size-difference groups. Experimental larvae were arranged to show similar (0-10%), a little different (10-20%) and completely different body size (over 20% difference in total body length). Numerals in parentheses, number of experimental tanks; vertical lines, ± SD. Statistical significance (using Mann-Whitney *U*-test) in the percent reduction of larvae between the siblings and mixed-siblings was indicated.

mixed-sibling groups were significant ($p < 0.05$ by Mann-Whitney *U*-test). Contrary to these, degrees of cannibalisms were identical between sibling and mixed-sibling groups with considerably different body size (over 20% difference in body length). Approximately 40 to 50 % of larvae were consumed by can-nibalisms in both, sibling and mixed-sibling groups. These observations suggested that the cannibalisms were induced even among mixed-sibling larvae with similar body size, and basically suppressed among sibling larvae with similar body size, but that the suppression of the cannibalisms among the siblings was overriden by extremely different body size of larvae. From these, it was concluded that preparing larvae with similar body size was very important to examine kinship recognition during the cannibalisms.

Identification of "non-cannibalistic" siblings

From the results of Experiment II and several preliminary observations, it was suggested that the larvae developed from each egg clutch were subdivided into two groups, "cannibalistic" and "non-cannibalistic", and thus analyzed in the following experiments.

Each of 20 larvae which had been hatched from different egg clutches respectively, was reared in separate plastic tanks, and the frequency of cannibalism was monitored everyday (Fig. 4). In 6 tanks out of 12 tanks, no cannibalisms occurred at all, and thus no reduction of larvae was observed through the experiment (25 days observation). Because the initiation of cannibalism in this species is not observed after 25 days of hatching and after the metamorphosis (Wakahara, 1995), these populations in the 6 tanks will never show cannibalisms throughout their lives. In the other 6 tanks, however, cannibalism were induced even among larvae from an egg clutch, and the number of larvae per tank reduced during early phase of the experiment. The cannibalism, whenever occurred, was initiated as early as 2 to 4 days after hatching. In other words, larvae developed from a specific clutch which had not shown cannibalism for the first 1 week of experiment, were expected not to show any kinds of cannibalisms at all thereafter.

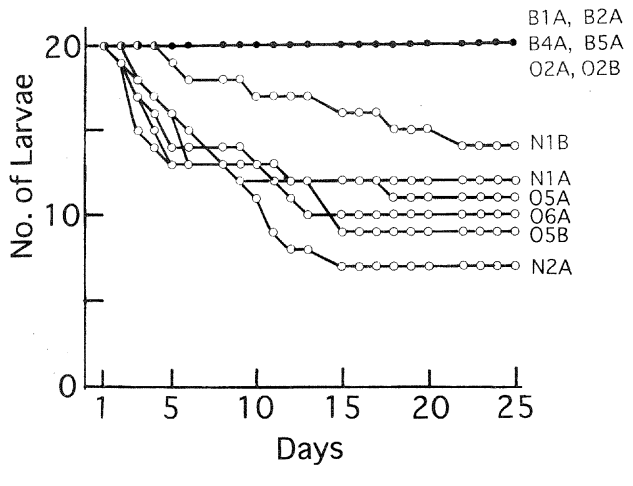

Fig. 4. Two patterns of reduction of number of larvae by cannibalisms in sibling groups. By rearing larvae developed from the same egg clutches for 10 days after hatching, they could be classified into two categories, either "cannibalistic" clutches which included the cannibals, or "non-cannibalistic" clutches which never showed the cannibalisms. Specifically, the larvae developed from the same egg clutches showed the identical behaviour, even though they were examined in different tanks, e.g., O2A and O2B ("non-cannibalistic"), N1A and N1B; N5A and N5B ("cannibalistic").

When the larvae hatched from one egg clutch were divided into two different tanks, the result whether cannibalisms were induced or not was identical in both tanks: if the cannibalism was induced in one tank, it was invariably observed in the other tank (e.g., N1A and N1B, O5A and O5B in Fig. 4), and if the cannibalism was not induced in one tank, it was not observed in the other (e.g., O2A and O2B). These observations were confirmed in another experiment using 340 larvae hatched from 17 different egg clutches (data not shown). Thus, it was possible to distinguish "cannibalistic" groups which contained the cannibalistic larvae from "non-cannibalistic" groups which never showed cannibalisms at all, by observing dynamics of each population hatched from different egg clutches for the first 10 days after hatching.

Cannibalism can be induced even in "non-cannibalistics"

In order to know whether members of the "non-cannibalistic" groups have an ability to eat the other individuals or not, the larvae which had been identified as "non-cannibalistics" were mixed with another "non-cannibalistic" larvae developed from different clutches. Newly hatched larvae from 40 different egg clutches were reared separately in 40 different tanks and cannibalisms were monitored for 10 days. As a result, 19 "non-cannibalistic" groups were recognized. After total body length of each larva was measured in order to control the body size-difference within 12.5%, twelve out of the 19 "non-cannibalistic" (NC) groups were selected for the experiment (Table 1). In 3 (tank no. 6, 7 and 8) out of 4 tanks which contained 30 to 40 "non-cannibalistic" larvae (NC alone), cannibalisms were not observed at all throughout the experimental period (25 days), but only one larva was eaten in one tank (tank no. 5). Contrary to these, in all 4 tanks that contained the similar number of larvae from two different "non-cannibalistic" groups (NC-NC combination, tank no. 1-4), a considerable number of larvae were eaten. Approximately 20% (19.7% ± SD12.9) of initial number of larvae were eaten in the experiment (NC-NC combination), significantly larger ($p < 0.01$ in Mann-Whitney *U*-test) than in the control (0.7% ± SD1.4, NC alone). Thus, the cannibals were induced even in the "non-cannibalistic" groups when larvae hatched from a different egg clutch were mixed with in the same tank.

Cannibalism in blinded larvae

In order to know what cues the larvae use to recognize sibship and avoid killing close relatives, and also to distinguish larvae from one clutch and ones from another clutch in one tank, eye balls were removed from larvae of one clutch and then mixed with intact larvae of either the same or another clutch (Table 2).

Table 1. Frequency of cannibalisms among "non-cannibalistic" larvae which were mixed with different egg clutches

Tank no.	Combination of larvae[1]	No. of larvae		No. of victims[2]	% Reduction (average ± SD)
		Initial	Final		
1	NC-NC	39	35	2	5.1
2	NC-NC	39	27	12	30.8
3	NC-NC	25	20	4	16.0
4	NC-NC	26	17	7	26.9 (19.7 ± 12.9)*
5	NC	34	30	1	2.9
6	NC	40	40	0	0.0
7	NC	33	32	0	0.0
8	NC	32	29	0	0.0 (0.7 ± 1.4)*

[1] Larvae developed from single clutches which had been identified as "non-cannibalistics" were mixed (NC-NC), or reared alone (NC).

[2] Initial number-(final number + dead larvae).

*$p < 0.01$ in Mann-Whitney U-test.

Table 2. Effect of eye removal on the recognition of kinship during the cannibalisms

Sibship(s)		% reduction of larvae by cannibalisms										
Two sibships	Tank no.	11	12	13	14	15	16	17	18	19	20	(average)
INT	(%)	40	71	34	40	44	25	36	83	40	56	46.9 *
ER[1]	(%)	31	53	0	14	27	43	8	0	13	0	18.9 *
Two sibships	Tank no.	21	22	23	24	25	26	27	28	29	30	(average)
INT[2]	(%)	11	0	15	0	9	33	0	19	20	32	13.9 **
ER	(%)	54	18	85	58	51	87	93	90	45	40	62.1 **
One sibship[3]	Tank no.	31	32	33	34	35						(average)
INT	(%)	0	0	0	0	0						0
ER	(%)	0	0	0	0	0						0
One sibship[4]	Tank no.	36	37	38	39	40						(average)
INT	(%)	38	52	15	36	45						37.2 ***
ER	(%)	30	78	20	32	53						46.6 ***

[1] Cannibals appeared in the eye-removed (ER) clutches.

[2] Cannibals appeared in the intact (INT) clutches.

[3] "Non-cannibalistic" clutches were chosen, a half of larvae were blinded (ER), and then mixed with intact (INT) larvae of the same clutches.

[4] Two cannibals from different clutches were introduced to the tanks where no cannibalisms had been observed.

*$p = 0.0180$ in Wilcoxon's signed ranks test.

**$p = 0.0051$ in Wilcoxon's signed ranks test.

***$p = 0.4185$ in Wilcoxon's signed ranks test.

Eye ball-removal made the larvae dark in their pigmentation, probably due to an excess release of MSH (Rowlands, 1952), and made easier to distinguish the sibships (Fig. 5). When the eye-removal operation was performed in the "cannibalistic" clutches (tank no. 11-20), they ate preferentially intact (i.e., non-kin) larvae: in average, 46.9% of the intact but only 18.9% of the blinded larvae were eaten ($p = 0.0180$ in Wilcoxon's signed ranks test). In 3 (tank no. 13, 18 and 20) out of 10 tanks, blinded cannibals exclusively ate intact (non-sibling), but avoided consuming blinded (sibling) larvae. Contrary to this, when the eye-removal operation was conducted in the "non-cannibalistic" clutches (tank no. 21-30), members of the intact larvae ate preferentially blinded larvae: in average, 62.1% of the blinded but only 13.9% of the intact larvae were eaten ($p = 0.0051$ in Wilcoxon's signed ranks test). In 3 (tank no. 22, 24 and 27) out of 10 tanks , intact cannibals exclusively ate blinded (non-sibling), but avoided consuming intact (sibling) larvae.

In one-sibship experiments, "non-cannibalistic" groups (such as O2 in Fig. 4) were chosen. Half of them were subjected to the eye ball-removal and then mixed with intact larvae developed from the same clutches. No cannibalisms were induced at all among the larvae of the same clutch, even though a half of them were blinded (tank no. 31-35). However, when cannibals from different clutches were added to their tanks (tank no. 36-40), they ate conspecifics irrespec-

Fig. 5. External views of blinded and intact “broad-headed” morph (cannibals), and blinded and intact “typical” morph (non-cannibals). The blinded larvae show darker pigmentation than the intanct probably due to an excess release of MSH.

tively either of intact or blinded: in average, 37.2% of the intact and 46.6% of the blinded larvae were eaten (not significant in Wilcoxon’s signed ranks test, $p = 0.4185$), suggesting that both intact and blinded larvae were consumed evenly. This means that the eye ball-removal itself had no effect on the cannibalism, either to consume or to be consumed.

DISCUSSION

Kinship recognition in amphibians has hardly been studied except for a few species such as a wood frog *Rana sylvatica* (Cornell *et al.*, 1989), a chorus frog *Pseudacris triseriata* (Smith, 1990), spadefoot toads *Scaphiopus couchii* (Newman, 1988) or *S. multiplicatus* (Pfennig, 1990), and the tiger salamander *Ambystoma tigrinum* (Pfennig and Collins, 1993). This is the first study to demonstrate convincingly that larvae of *Hynobius retardatus* can recognize kinship without sence of sight and preferentially consume non-kins or avoid killing siblings (Table 2). Thus, the cannibalism in *H. retardatus* may provide a new material for investigation of the kinship recognition in amphibians.

Cannibalistic larvae in *Hynobius retardatus* develop to a “broad-headed” morph (Wakahara, 1995) and grow faster compared with a non-cannibalistic “typical” morph (Fig. 1) like in Arizona tiger salamander (*Ambystoma tigrinum neburosum*) (Collins and Cheek, 1983; Collins and Holomuzki, 1983; Pfennig and Collins, 1993). The faster development, earlier transition to terrestrial habitats and larger body size of the cannibals will reflect to their higher reproductive success which must be balanced with the costs of the cannibalism, ie., risk of injury or death (Ohdachi, 1994), eating relative (Crump, 1992) or infection from the victim (Pfennig *et al.*, 1991). The problem is how the larvae recognize kinship, avoid killing siblings and increase their inclusive fitness.

The cannibals in the Arizona tiger salamander are reported to be induced facultatively by high densities of conspecifics and low food supply, and will be suppressed among pure sibships (Pfennig and Sherman, 1995). In *Hynobius retardatus* also cannibalisms were induced more frequently in high density of larvae and low food level, and less frequently among sibling larvae (Wakahara, 1995). Results described in this study support the previous observations and suggest that the larvae of these species recognize kinship and that even the cannibal morph avoids killing siblings when given a choice between siblings and non-kin, and preferentially eats unrelated larvae (Pfennig and Collins, 1993). These kin-recognition mechanism effectively remove the potential loss of inclusive fitness that arizes by cannibalizing close kin (Sadler and Elgar, 1994).

In the larvae of the American toad *Bufo americanus*, olfactory basis of kin recognition was demonstrated: tadpoles whose external nostrils were blocked with a gelatinous paste did not discriminate between siblings and non-siblings, while they could discriminate siblings from non-siblings after their nostrils were unplugged (Waldman, 1985). It is also hypothesized that expression of the cannibal morph is influenced by sibship-specific olfactory signal in Arizona tiger salamander (Pfennig and Collins, 1993; Pfennig *et al.*, 1994). Cannibals with blocked nostrils were unable to discriminate between different larvae (Pfennig *et al.*, 1994). Sibship-specific chemical cues seem to cause certain frog tadpoles to grow faster in water conditioned by kin as opposed to non-kin. Indeed, tadpoles of several anuran species grow larger when reared in pure-sibship groups than when reared in mixed-sibship groups (Smith, 1990). In *Hynobius*, chemical messenger might affect the morphogenesis of head and oral structure as well (Nishihara, 1996). Morphogenesis may be responsive to kinship in this species that facultatively develops structures that can be used against conspecifics as weaponry (Pfennig and Collins, 1993). Thus, sibship-specific chemical cues seem to suppress to induce cannibal-morph, resulting in avoiding killing siblings. At present, however, chemical nature and/or molecular basis of the chemical cues or olfactory signals have not been identified yet in any amphibian species.

Because a lot of males spawn spermatozoa simultaneously to a pair of egg sacs spawned by one female during the reproduction of this species (Sasaki, 1924), sibling larvae are genetically divided into either full sib in one case or half sib in the other. This may correlate with the presence of two types of siblings, “non-cannibalistic” and “cannibalistic” ones: possibly cannibalisms are suppressed or not induced among the full sib, but are not suppressed or induced in the half sib larvae. Further analyses using pure sibship larvae developed from artificially inseminated eggs are necessary to elucidate more accurate relationship between the occurrence of cannibalism and kinship.

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