

## Absence of kin discrimination in cannibalistic Anuran tadpoles of the frog *Hoplobatrachus tigerinus* (Daudin)

Amrapali P Rajput, Bhagyashri A Shanbhag\* & Srinivas K Saidapur

Department of Zoology, Karnatak University, Dharwad 580 003, India

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Kin discrimination was tested in the cannibalistic *H. tigerinus* tadpoles to know whether cannibalism is selectively directed towards non-kin members or it is indiscriminate. The association choice tests were conducted using satiated as well as starved subjects with the assumption that they will associate near non-sibs rather than near sibs with the intention of preferentially cannibalizing them. However, test tadpoles, fed or starved showed a random association choice with sibs and non-sibs, as in the end-bias stimulus blank tests. Therefore it is suggested that cannibalistic *H. tigerinus* tadpoles do not discriminate sibs from non-sibs and cannibalize on both rather indiscriminately.

**Keywords:** Behaviour, Cannibalism, Frog tadpoles, *Hoplobatrachus tigerinus*, Kin discrimination

The ability to discriminate kin from non-kin is studied in several species of amphibian tadpoles<sup>1-7</sup>. It is generally believed that such kin recognition in amphibian tadpoles is beneficial in many ways conferring inclusive fitness in these species. However, kin recognition is not universal even among the amphibian larvae. Moreover, the larvae of several species of amphibians are known to be cannibalistic<sup>8</sup> which in a way necessitates kin discrimination. Indeed, kin discrimination is reported in the cannibalistic larvae of salamanders, *Ambystoma opacum*<sup>9</sup>, *Ambystoma tigrinum nebulosum*<sup>10,11</sup>, and *Hynobius leechii*<sup>12</sup>. However, kin discrimination in these species is reported to be context dependent. For instance, kin recognition was less pronounced when *A. tigrinum* co-occur with other species of salamanders<sup>11</sup>. Interestingly the larvae of Korean salamander, *H. leechii* are reported to preferentially cannibalize on small sibs than small non-sibs in a mixed group<sup>12</sup>. Among cannibalistic anuran tadpoles, spadefoot toad tadpoles, *Scaphiophrynus* discriminate between sibs and non-sibs, but more hungry individuals eat their sibs also<sup>13</sup>. The study showed that the level of kin discrimination in these tadpoles is dependent on the level of their hunger<sup>13</sup>. In contrast, tadpoles of the green poison frog, *Dendrobates auratus* are indiscriminate predators, and they cannibalize both sibs and non-sibs<sup>14</sup>.

The Indian bull frog, *Hoplobatrachus tigerinus* (Daudin) is an explosive breeder. It breeds in ephemeral ponds. The oral disc of *H. tigerinus* tadpoles is well suited for carnivory<sup>15</sup>. These tadpoles are also cannibalistic in nature<sup>16</sup>. In laboratory, when raised with kin in large densities they are observed to cannibalize on sibs (personal observation). However, it is not known whether they can discriminate between sibs and non-sibs, given an opportunity in a mixed population whether these tadpoles avoid eating sibs. Hence experiments have been designed to know whether *H. tigerinus* tadpoles show kin discrimination and thereby avoid sib cannibalism.

In kin recognition study, association choice of test tadpoles is considered to reflect their ability to recognize sibs and non-sibs. However, unlike in kin recognition studies, it is hypothesized that association choice of cannibalistic *H. tigerinus* tadpoles near non-sibs and avoidance of sibs would indicate their ability to discriminate kin from non-kin since going near the prey indicates predator's intention to consume the prey. This would also reveal their intention to feed selectively on non-sibs rather than sibs. Hence, trials have been designed using hungry or well fed test tadpoles of *H. tigerinus* to know their choice of association with sibs vs non-sibs. It is assumed that the test tadpoles would not associate with sibs if they have kin discrimination ability regardless of their hunger levels. The study thus examines the effect of kinship on sib cannibalism avoidance behaviour if any, in the tadpoles of *H. tigerinus* through kin discrimination trials.

\*Correspondent author  
Telephone/Fax: 91-836-2440190  
E-mail: bhagyashrishanbhag@gmail.com

## Materials and Methods

Eggs from five clutches of *H. tigerinus* were collected in the month of May from different ephemeral ponds located in the University Campus, (Latitude 15° 17' N, Longitude 75° 3' E). Eggs from each clutch were placed in a separate plastic tub containing 10 L aged tap water. The eggs hatched within three days of collection. Around 150 tadpoles (Gosner stage 19) from each parental line were reared in sib groups in rectangular glass aquaria (75×45×15 cm) containing 25 L aged tap water. On reaching Gosner stage 25 they were provided with tadpoles of *Bufo melanostictus* as food/prey. Since cannibalism was observed in the rearing regimes, prey tadpoles of *B. melanostictus* were provided *ad libitum* and this prevented cannibalism. The work was conducted as per the guidelines laid down by CPCSEA, New Delhi.

A rectangular test tank (120×30×18 cm) made of glass was used to conduct test trials; 15 cm long two end compartments were created by placing 2 mm thick transparent perforated acrylic mesh partitions. Area between the mesh partitions on the either sides and the center of the tank were designated as zone A and B and served as the test arenas. Required number of tadpoles from each parental line was kept individually in tubs (20×6 cm) for 24 h prior to trials and they were not fed. These tadpoles served as starved test tadpoles. The end compartments housed stimulus tadpoles (n=20) or kept stimulus blank (empty) as per the experimental protocol. For each trial, a single test tadpole (stage 27-30), starved (24 h) or well fed, was introduced into an open ended mesh cage (10 cm dia.) placed in the center of the test tank. It was held for 10 min in the cage before release. The cage was gently lifted to allow free movement of the test tadpole. No recordings were made for one minute after release. As a measure of association preference, time (s) spent by the test tadpole, and number of test tadpoles spending maximum time in each zone were recorded for 10 min. A given test tadpole was used once only. The test tank was thoroughly cleaned and filled with aged tap water (3 cm) before each trial. Stimulus tadpoles (n=20) were renewed after 10 trials and their position in the test tank was reversed between trials. Developmental stage and size of the test and stimulus tadpoles were always matched. The following tests were conducted.

End-bias tests were conducted to check the potential bias of test tadpoles towards any side of the test tank in the absence of any stimulus tadpoles. Random distribution of test tadpoles is considered to

indicate non-bias towards any side of the tank. 50 trials were conducted (10/ parental line).

Association preference of test tadpoles with familiar sibs and unfamiliar non-sibs was studied using fed individuals. In these trials, the end compartments housed 20 stimulus tadpoles belonging to two different parental lines. Stimulus tadpoles housed in one of the end compartments were sibs of test tadpoles and thus familiar to them. The other end compartment housed unfamiliar non-sibs. It is hypothesized that the cannibalistic *H. tigerinus* test tadpoles would move randomly between zones housing familiar sibs and unfamiliar non-sibs as they were satiated. A total of 100 trials were conducted, using tadpoles from 5 different parental lines.

In the second set of trials, association preference of starved tadpoles with familiar sibs and unfamiliar non-sibs was studied. The experimental set-up was similar to the above design except that the test tadpoles were starved. It is assumed that the hungry test tadpoles would prefer to cannibalize non-sibs over sibs and therefore associate themselves with non-sibs rather than with sibs, if they discriminated kin and non-kin. In this test also 100 trials were conducted.

The number of tadpoles spending majority of their time in each stimulus zone was compared using binomial tests. Wilcoxon signed-rank test was used to analyze the time spent by test tadpoles near sibs and non-sibs. Differences in the time spent near the respective zones of the test tank were tested from a hypothetical random distribution expected under the null hypothesis that the test tadpoles would spend equal amount of time (300 s) in each test arena. Therefore, only one score per animal was used in comparison with the expected mean not to violate the independence of data. All tests were two tailed. The data were judged to be significant at  $\alpha < 0.05$ . Meta analysis of different test groups of the same rearing type was performed using Fisher's procedure of combining probabilities from independent tests of significance for an overall result<sup>17</sup>. Fisher's procedure was applied on probabilities obtained from binomial test and Wilcoxon signed-rank test. Comparison was also made between number of tadpoles of starved and fed groups spending majority of their time near sibs and non-sibs using Mann-Whitney *U*-test.

## Results and Discussion

In end-bias tests the test tadpoles moved randomly throughout the test arena indicating their non-bias

towards any side of the test tank (Table 1). The fed test tadpoles when given a choice between familiar sibs and unfamiliar non-sibs, showed random distribution showing no significant difference in the time spent by them near sibs or non-sibs. There was no discrimination in the association choice between the two stimulus groups (Fig. 1A). Similarly, when starved test tadpoles were given a choice to associate with familiar sibs or unfamiliar non-sibs, their distribution in the test tank was also random depicting no preference for either stimulus group tadpoles (Fig. 1B). Also no significant difference was found between number of fed and starved tadpoles, spending most of their time near sibs and non-sibs.

Among the cannibalistic larval amphibians, kin discrimination has been studied mainly in cannibalistic larval salamanders<sup>4,9,11,12,18</sup> while corresponding studies on cannibalistic anuran tadpoles are limited to a few species<sup>5,13,14</sup>. The spade-foot toad tadpoles, *Spea bombifrons* and *S. multiplicata* exhibit polyphenism, in which omnivorous tadpoles associate preferentially with sibs whereas carnivorous tadpoles avoid sibs<sup>5,13</sup>. Nevertheless, kin discrimination in the carnivorous spade-foot toad tadpoles is context dependent<sup>5</sup>. They avoid eating sibs when well fed, but do not care to discriminate between sibs and non-sibs when food is scarce. Thus a lack of cannibal's ability to discriminate between kin and non-kin and resort to indiscriminate cannibalism (involving kin and non-kin) may still be favored when the cost of not feeding on kin would threaten their own survival. Thus, kin cannibalism in these tadpoles is possibly selected against kin discrimination under low nutrient condition when cost of kin discrimination would actually override the benefits of inclusive fitness. In contrast, the absence of kin discrimination is reported in the poison frog, *D. auratus* tadpoles which cannibalize sibs and non-sibs indiscriminately<sup>14</sup>. It is not surprising to know that the

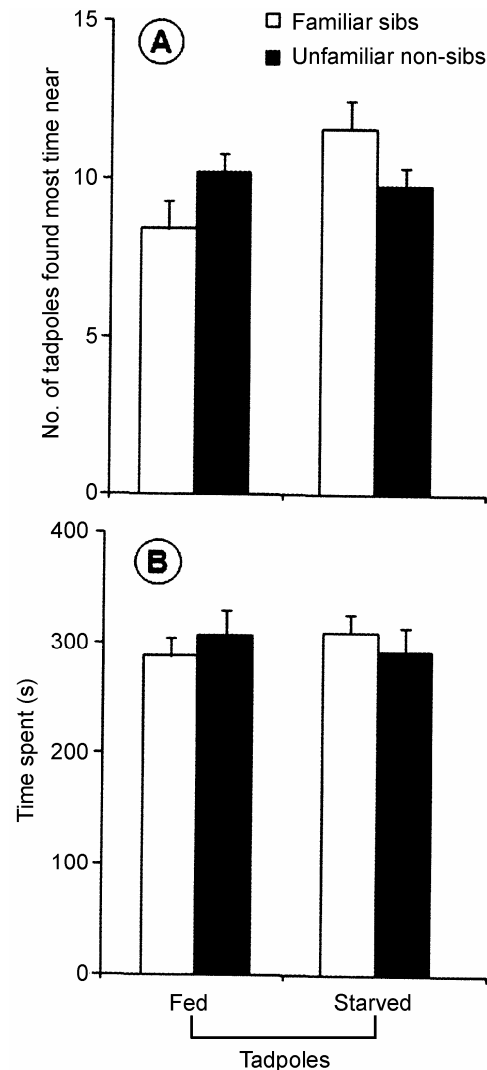


Fig.1—Number of fed or starved *H. tigerinus* tadpoles associating near zones with (A) familiar sibs and unfamiliar non-sibs and (B) time (s) spent with familiar sibs and unfamiliar non-sibs (values are mean  $\pm$  SE).

tadpoles of *D. auratus* do not have kin discrimination system/mechanism considering the habitat in which they live. These tadpoles develop in small pools of water (phytotelmata) facing high risk of drying up, having low nutrient supply and high predation pressure. Such harsh ecological conditions may necessitate the feeding on the pool-mates to derive needed nutrients and nourishment for growth and survival till metamorphosis<sup>19</sup>. Therefore, selection may not have favored kin discrimination in tadpole species that live in small and nutrient poor environment associated with high level of competition that make kin discrimination a costly affair. These studies suggest that evolution of kin cannibalism is perhaps context based driven by ecological conditions faced by the

Table 1—Distribution of *H. tigerinus* tadpoles in end-bias tests

Parental line	Number spending most time near <sup>a</sup>		Time spent (s) in zone <sup>b</sup> (mean $\pm$ SE)	
	Zone A	Zone B	Zone A	Zone B
P <sub>1</sub>	03	04	259.50 $\pm$ 77.13	340.50 $\pm$ 77.13
P <sub>2</sub>	04	06	290.80 $\pm$ 74.00	309.20 $\pm$ 74.00
P <sub>3</sub>	07	03	388.60 $\pm$ 69.03	211.40 $\pm$ 69.03
P <sub>4</sub>	07	03	259.70 $\pm$ 56.55	340.30 $\pm$ 56.55
P <sub>5</sub>	05	05	324.30 $\pm$ 60.09	275.70 $\pm$ 60.09

<sup>a</sup>Compared using binomial test ; <sup>b</sup> compared using Wilcoxon signed-rank test; Fisher's procedure of combining probabilities for overall result:  $-2\sum \ln P = 8.56$ ,  $\chi_{(10)}^2 P > 0.05$  (number data);  $-2\sum \ln P = 4.96$ ,  $\chi_{(10)}^2 P > 0.05$  (time data).

larvae. The results of the present study show that tadpoles of *H. tigerinus* do not discriminate between kin and non-kin regardless of hunger level (fed or starved). Also, in the rearing regime, if the food was not in plenty cannibalism was seen in sib group. Given a chance they cannibalize sibs and non-sibs equally. These observations are in agreement with those on *D. auratus* that do not discriminate between kin and non-kin<sup>14</sup>. However, tadpoles of *H. tigerinus* are found in ephemeral water bodies formed during rainy season. Perhaps, their habitat is comparable to those of spadefoot toad tadpoles and not that of *D. auratus* tadpoles. Therefore it is difficult to say why selection has favored kin cannibalism against kin selection in *H. tigerinus* based on the present laboratory based study. Additional studies are needed to understand evolution of kin cannibalism and lack of kin discrimination in *H. tigerinus* tadpoles.

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