Association preference and mechanism of kin recognition in tadpoles of the toad *Bufo melanostictus*

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In experiments with specially designed choice tanks, tadpoles of *Bufo melanostictus* spend significantly greater amounts of time near kin than near non-kin. However, in the absence of kin members, they prefer to spend more time near non-kin rather than stay away in isolation in the opposite blank zone with no company. This implies that association of toad tadpoles with their kin is due to attraction rather than repulsion from non-kin. Experiments designed to elucidate the sensory basis of kin recognition showed that toad tadpoles recognize their kin based on chemical cues rather than visual cues. They can also discriminate between homospecific non-kin and heterospecific (*Sphaerotheca breviceps*) tadpoles since the tadpoles spent significantly greater amounts of time near the former than near the latter. These findings suggest that where kin members are unavailable, selection may have favoured living with non-kin so as to derive benefits from group living and that a phenotype-matching mechanism may operate for both kin and species discrimination in *B. melanostictus.*

1. Introduction

Kin recognition is a widespread phenomenon in the animal kingdom (Pfennig and Sherman 1995; Holmes 2004) as well as in plants (references in Dudley and File 2007). Among amphibians, tadpoles of some species of frogs and toads are well known to exhibit kin recognition abilities (Blaustein 1988; Waldman 1986, 1991; Saidapur and Girish 2000). However, the exact mechanism of kin recognition in anuran tadpoles is far from clear as studies on this aspect are limited. It is generally believed that kin recognition in anuran tadpoles is done by phenotypic matching or through recognition alleles or based on familiarity (Waldman 1987; Blaustein 1988). Yet, the genetics and neurophysiology of kin recognition mechanisms continue to exist largely as black boxes (Holmes 2004).

Bufo melanostictus breed both singly and communally in ephemeral ponds. The tadpoles of this toad are gregarious. Depending upon the circumstances, they may live as members of kin and/or mixed groups until metamorphosis (Saidapur and Girish 2000). A previous study has shown that the toad tadpoles recognize their kin throughout larval development and, given a choice, prefer to associate with unfamiliar kin rather than familiar non-kin (Saidapur and Girish 2000). However, the sensory basis of such a recognition system (e.g. visual or chemical) is unknown.

A preferential association with kin over non-kin may also result following repulsion by signals from non-kin, forcing the tadpoles to associate with their kin. Therefore, it is of interest to know how an individual tadpole would behave in the presence of exclusively non-kin; would it prefer to join a conspecific non-kin group or remain in isolation? Further, it is of great interest to know whether the toad tadpoles can also discriminate between homospecific non-kin and heterosopecific tadpoles. The present study was designed to answer these questions.

2. Materials and methods

Five egg masses of *B. melanostictus* were collected on 25 and 30 June 2007 from a local park in Dharwad city (latitude

15°7′N, longitude 75°3′E). The eggs of each clutch were placed separately in glass tanks containing 10 l of aged (dechlorinated) tap water. From stage 25 (feeding stage, Gosner 1960) onwards, the tadpoles of a given clutch were reared together in plastic tubs of 5 l capacity to familiarize themselves with their siblings. They were fed with boiled spinach on alternate days except during the trial periods. Tadpoles of stage 28–30 were used for the test trials. Two sets of experiments were conducted to elucidate (i) the association preference of test tadpoles when given a choice between a non-kin group and a blank zone, and (ii) the possible basic mechanism of kin recognition (visual or chemical).

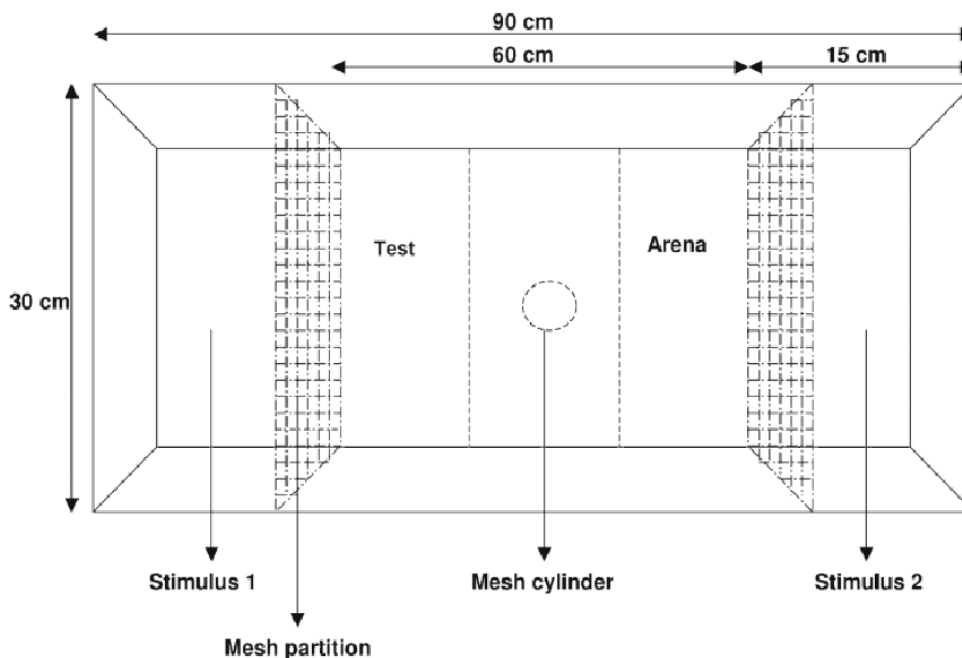### 2.1 *Design of the choice tank and experimental protocol*

A rectangular glass aquarium measuring 90 x 30 x 15 cm was used as the choice tank. At the opposite ends of the choice tank, 2 mm thick, perforated, transparent acrylic sheet partitions with 1 mm diameter holes (~6/cm², total 1290 holes) were placed to create 15 cm end compartments (figure 1) and used to house kin or non-kin stimulus tadpoles. In the absence of any tadpoles, the compartment served as the stimulus-blank zone. The central area (60 cm) served as the test arena. Two lines perpendicular to the long axis were drawn on the outer side at the bottom of the tank to divide the central area such that the central zone was 20 cm in length and adjacent zones of 20 cm length were created.

Test tadpoles were released in the middle of the central compartment while zones adjacent to it, separated by the perforated acrylic sheets from the end compartments, served as stimulus zones. A given test tadpole was introduced in an open-ended mesh cage (9 cm in diameter), placed in the middle of the central compartment and held for 10 min to allow the subject to adjust to the choice tank before releasing it by gently lifting the cage. The test tadpole could then swim freely to familiarize itself with the test arena and associate with the stimulus zone of its choice. In all trials, the same protocol was followed.

As a measure of association preference, we recorded the time spent by a test tadpole in each stimulus zone during the trial period of 10 min after its release from the cage, using a stop watch. The tank and the perforated acrylic sheet partitions were washed after each trial. The stimulus compartments were reversed between trials. Stimulus tadpoles were renewed after every 10 trials. Ten replicates were performed per parental line in each type of test to give a total of 50 trials in each group.

2.2 *End-bias tests*

End-bias tests were conducted to rule out bias of tadpoles, if any, towards a particular side of the choice tank, the stimulus-blank end zones. It is hypothesized that distribution of test tadpoles in the absence of stimulus tadpoles will be random and exhibit no bias towards any particular side of the

Figure 1. Design of the choice tank (top view) used in experiment 1 to study the association preference of *B. melanostictus* tadpoles with kin over stimulus blank or kin over non-kin or non-kin over stimulus-blank zones.

choice tank. In the second set of end-bias tests, depending upon the experiment, the end zones housed a transparent glass beaker or open-ended mesh cage with or without a cheesecloth wrapper.

2.3   *Experiment 1. Association preference of toad tadpoles with kin, non-kin and blank zone under different situations*

In this experiment, the following tests for association choice were conducted.

2.3.1   *Association preference with kin and blank zone*: In this test, siblings were housed in one of the end compartments and the opposite end compartment served as the blank. This test was designed to find out whether test tadpoles prefer to stay near sibs in the absence of any other stimulus in the opposite zone of the test tank.

2.3.2   *Association preference with kin and non-kin*: In this test, siblings were housed in one of the end compartments and non-siblings in the opposite end compartment. These tests were conducted as a baseline for the present study though kin recognition has previously been reported in tadpoles of *B. melanostictus* (Saidapur and Girish 2000).

2.3.3   *Association preference with non-kin and blank zone*: In this test, non-siblings were housed in one of the end compartments and the opposite end compartment served as the blank. This test was designed to find out whether the test tadpoles are actually repelled by the stimulus cues of non-siblings. If so, test tadpoles would avoid associating with non-siblings and occupy the blank zone.
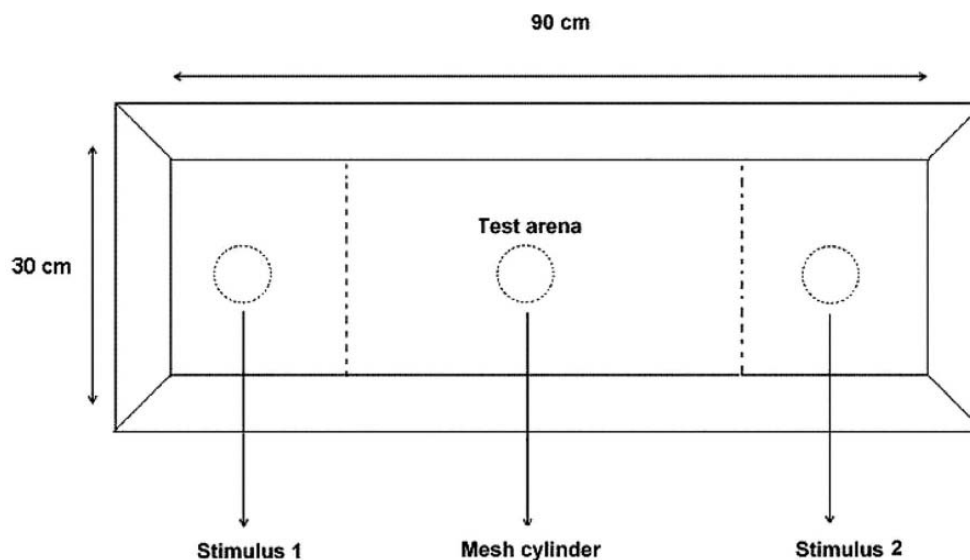
2.4   *Experiment 2. Mechanism of kin recognition*

This experiment was conducted to know whether test tadpoles recognize their siblings based on visual or chemical cues. The choice tank used was of the same type as in experiment 1 but it was not partitioned (figure 2). In the end zones, circles (10 cm in diameter) were drawn at the outside bottom of the tank equidistant from the centre of the tank and were used to place the glass beaker/mesh cage housing the stimulus tadpoles. The experimental design consisted of housing tadpoles in the end zones, in a glass beaker (permitting visual cues) or in an open-ended mesh cage (9 cm in diameter) wrapped with cheesecloth (permitting the diffusion of chemical cues). In addition to end-bias tests, the following tests were also carried out.

2.4.1   *Recognition based on only visual cues*: In this test, siblings and non-siblings were housed at opposite ends of the choice tank inside transparent glass beakers. If tadpoles recognize their sibs by visual cues, they would spend more time near the beaker housing their sibs.

2.4.2   *Recognition based on only chemical cues*: In this test, siblings and non-siblings were placed at opposite ends of the choice tank inside two mesh cylinders wrapped with cheesecloth. Test tadpoles were then tested for their choice of association. If tadpoles recognize their sibs based on perception of chemical cues, they would prefer to spend more time near the mesh cylinder covered with cheesecloth housing the sibs.

2.4.3   *Association preference with kin: use of chemical vs visual cues*: In this test, siblings were housed either in a glass beaker or a mesh cage wrapped with cheesecloth placed at the opposite ends of the choice tank. Test tadpoles were thus exposed to both visual and chemical cues of their

Figure 2.   Design of the choice tank (top view) used in experiment 2 to understand the sensory basis of the kin recognition mechanism.

siblings, which allowed them to reveal the nature of sensory perception used in kin recognition.

2.4.4 *Association preference with non-kin or heterospecifics based on chemical cues*: In the above experiments, test tadpoles exhibited a clear preference for siblings over non-siblings. They also showed a preference towards non-siblings when siblings were unavailable. Hence, this experiment was designed to determine whether toad tadpoles prefer to associate with non-siblings in the presence of heterospecific tadpoles and thus provide proof of discrimination between conspecifics and heterospecifics. Therefore, in this experiment, non-sibling tadpoles of *B. melanostictus* (homospecific) were housed at one end and *Sphaerotheca breviceps* tadpoles (heterospecific) at the opposite end of the choice tank; both groups were housed in mesh cylinders wrapped with cheesecloth.

2.5 *Statistical analysis*

The mean time spent by test tadpoles (from each parental line) near a particular stimulus zone was analysed by the Wilcoxon signed-rank test. We tested the time spent by test tadpoles in a particular stimulus zone from a hypothetical time expected (200 s) under the null hypothesis that tadpoles would spend equal amounts of time in each zone of the test arena. Therefore, only one score was used to compare with the expected mean so as to not violate the independence of the data.

For each parental line, the number of tadpoles that spent a majority of time in different stimulus zones was compared using the binomial test. Meta-analysis of data from different parental lines belonging to a test group was performed with the Fisher procedure to combine probabilities (Sokal and Rohlf 1995) from independent tests of significance for an overall result. The Fisher procedure was applied on probabilities obtained from the binomial test and Wilcoxon signed-rank tests. The data from 50 trials (10 trials/parental

line) for each test are represented by box whisker plots to provide dispersion patterns.

3. Results

3.1 *End-bias test*

In the end-bias test, there was no significant difference in the time spent by test tadpoles in either side of the choice tank, implying that they show no bias towards the end compartments in the absence of stimulus cues (table 1, figure 3A). Overall analysis of the number of tadpoles who spent their time at either end of the test tank showed no significant variation (table 1). The results of the other three tests are given below.

3.2 *Experiment 1. Association preference of toad tadpoles with non-kin in the absence of either kin at the other zone or blank zone*

3.2.1 *Association preference with kin and blank zone*: Tadpoles spent significantly greater amounts of time near stimulus zones housing siblings than near the stimulus-blank zones (table 2, figure 3B). Also, a significantly larger number of tadpoles spent a majority of time in the zone housing siblings than in the blank zone (table 2).

3.2.2 *Association preference with kin and non-kin*: Tadpoles spent significantly more time near the stimulus zones housing siblings than near non-siblings (table 3, figure 3C). Also, a significantly larger number of tadpoles spent the majority of time near siblings rather than near non-siblings (table 3).

3.2.3 *Association preference with non-kin and blank zone*: In these tests, test tadpoles spent more time near non-siblings than near the blank zone (table 4, figure 3D). Furthermore, the number of tadpoles spending most of their time near non-siblings was significantly greater compared with those in the blank zone (table 4).

Table 1. Association preference of test tadpoles (*B. melanostictus*) in end-bias tests with reference to stimulus zones of the choice tank

| Parental line | Number spending most[a] time near | | Time (s) spent in zones[b] (mean ± SE) | |
	Zone A	Zone B	Zone A	Zone B
P1	7	3	278.00 ± 32.59	207.90 ± 32.09
P2	6	4	241.70 ± 20.48	246.10 ± 20.51
P3	4	6	219.50 ± 14.95	288.00 ± 22.37
P4	6	4	248.90 ± 25.62	254.80 ± 32.32
P5	4	6	258.20 ± 32.44	242.30 ± 28.81

[a]Compared using binomial test. [b]Time spent in stimulus zones was compared using a random expectation by the Wilcoxon signed-rank test.

Fisher test of combining probabilities used for overall results: $-2\sum \ln P = 4.38$, $\chi^2[10] : P > 0.05$ (number data); $-2\sum \ln P = 8.688$, $\chi^2[10] : P > 0.05$ (time data).
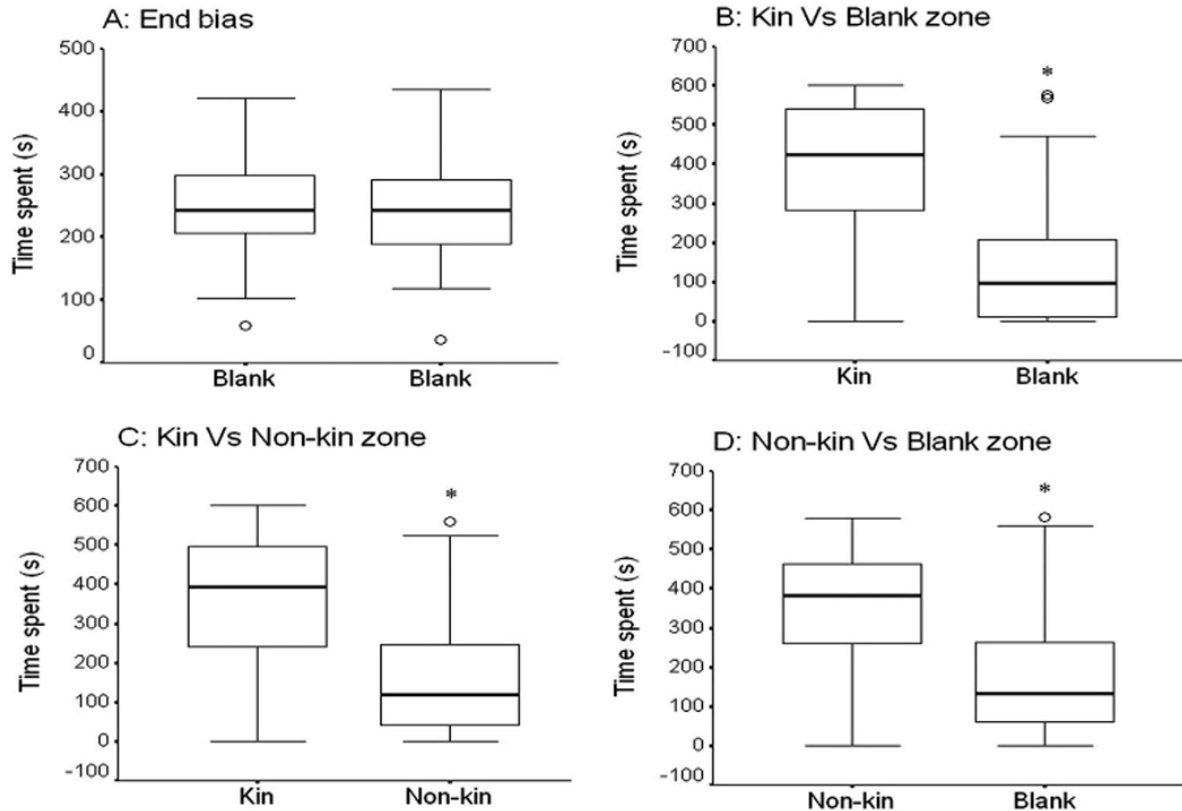
Figure 3. Box whisker plots depicting the association preference of test tadpoles for zones A and B. Boxes represent interquartile ranges. Horizontal bars in the boxes represent medians, whiskers represent the farthest data points that are not outliers, open circles above the whiskers represent outliers. The asterisks denote a significant difference in time spent by test tadpoles between the two zones. **(A)** End-bias test – both stimulus zones blank. **(B)** Tests with kin vs blank. **(C)** Tests with kin vs non-kin. **(D)** Tests with non-kin vs blank.

Table 2. Association choice of *B. melanostictus* tadpoles between kin and stimulus-free zones of the choice tank (experiment 1)

	Number spending most[a] time near		Time (s) spent in zones[b] (mean ± SE)	
Parental line	Kin	Blank zone	Kin	Blank zone
P1	7	3	334.40 ± 37.63*	156.30 ± 31.27
P2	10*	0	438.40 ± 34.78*	97.30 ± 26.83
P3	9*	1	438.20 ± 43.70*	104.40 ± 37.98
P4	7	3	338.90 ± 67.13	190.20 ± 68.68
P5	9*	1	395.00 ± 58.77*	149.10 ± 47.55

[a] Compared using binomial test. [b] Time spent in stimulus zones was compared using a random expectation by the Wilcoxon-signed rank test. *Significantly different. Fisher test of combining probabilities used for overall results: $-2\sum \ln P = 34.64$, $\chi^2 [10]$: $P<0.001$ (for number data); $-2\sum \ln P = 18.604$, $\chi^2 [10]$: $P<0.05$ (time data).

3.3 *Experiment 2. Mechanism of kin recognition*

In the end-bias tests with a glass beaker or a mesh cage wrapped with cheesecloth placed at the opposite ends, there was no significant difference in the number of or time spent by test tadpoles on either side of the choice tank (table 5, figure 4A).

3.3.1 *Recognition based on only visual cues*: There was no significant difference in the number of test tadpoles and time spent by them near siblings or non-siblings housed in glass beakers (table 6, figure 4B).

3.3.2 *Recognition based on only chemical cues*: The time spent by the test tadpoles near siblings was significantly greater than that near non-sibling stimulus tadpoles housed

Table 3. Association choice of *B. melanostictus* tadpoles between kin and non-kin zones of the choice tank (experiment 1)

Parental line	Number spending most[a] time near		Time (s) spent in zones[b] (mean ± SE)	
	Kin	Non-kin	Kin	Non-kin
P1	6	4	329.20 ± 36.64	166.10 ± 54.37
P2	7	3	305.70 ± 28.19*	154.60 ± 52.12
P3	7	3	363.10 ± 48.55*	208.60 ± 54.93
P4	9*	1	361.50 ± 24.98*	146.20 ± 39.80
P5	9*	1	403.40 ± 32.92*	134.60 ± 50.32

[a] Compared using binomial test. [b] Time spent in stimulus zones was compared using a random expectation by the Wilcoxon signed-rank test. *Significantly different. Fisher test of combining probabilities used for overall results: $-2\sum \ln P = 20.14$, $\chi^2 [10] : P<0.05$ (number data); $-2\sum \ln P = 20.328$, $\chi^2 [10] : P<0.05$ (time data).

Table 4. Association choice of *B. melanostictus* tadpoles between non-kin and stimulus-free zones of the choice tank (experiment 1)

Parental line	Number spending most[a] time near		Time (s) spent in zones[b] (mean ± SE)	
	Non-kin	Blank zone	Non-kin	Blank zone
P1	9*	1	407.10 ± 29.85*	126.70 ± 28.66
P2	9*	1	351.20 ± 30.76*	167.00 ± 51.59
P3	5	5	319.80 ± 43.84	202.50 ± 57.64
P4	8	2	323.80 ± 34.96*	188.10 ± 29.66
P5	7	3	326.10 ± 38.68*	171.50 ± 40.99

[a] Compared using binomial test. [b] Time spent in stimulus zones was compared using a random expectation by the Wilcoxon-signed rank test. *Significantly different. Fisher test of combining probabilities used for overall results: $-2\sum \ln P = 18.74$, $\chi^2 [10] : P<0.05$ (number data); $-2\sum \ln P = 19.10$, $\chi^2 [10] : P<0.05$ (time data).

Table 5. Association preference of test tadpoles (*B. melanostictus*) in end-bias tests with reference to stimulus zones (devoid of tadpoles) of the choice tank (experiment 2)

Parental line	Number spending most[a] time near		Time (s) spent in zones[b] (mean ± SE)	
	Zone A	Zone B	Zone A	Zone B
P1	5	5	262.7 ± 28.88	253.3 ± 32.82
P2	5	5	244.1 ± 24.74	242.3 ± 27.30
P3	6	4	249.6 ± 32.11	220.6 ± 24.52
P4	6	4	256.4 ± 30.86	253.1 ± 36.33
P5	5	5	274.9 ± 28.95	266.5 ± 26.30

[a] Compared using binomial test. [b] Time spent in stimulus zones was compared using a random expectation by the Wilcoxon signed-rank test.
Fisher test of combining probabilities used for overall results: $-2\sum \ln P = 1.12$, $\chi^2 [10] : P>0.05$ (number data); $-2\sum \ln P = 9.43$, $\chi^2 [10] : P>0.05$ (time data).

in a mesh cage wrapped with cheesecloth (table 7, figure 4C). Also, a significantly larger number of test tadpoles resided near the zone housing siblings though they could not be seen (table 7).

3.3.3 *Association preference with kin: use of chemical vs visual cues*: In these tests, siblings (stimulus tadpoles) housed in the glass beaker provided visual cues and those housed in the mesh cage wrapped with cheesecloth provided chemical cues. Test tadpoles spent significantly more time near siblings housed in the mesh cage covered with cheesecloth than near the glass beaker containing their siblings (table 8,

figure 4D). The number of tadpoles that spent a majority of time near siblings housed in the mesh cage was significantly larger than those found near the glass beaker.

3.4.4 *Association preference with non-kin or heterospecifics based on chemical cues*: In this experiment, two stimulus groups, homospecific non-siblings and heterospecific (*S. breviceps*) tadpoles, were housed in mesh cages covered with cheesecloth and placed at opposite ends of the choice tank. Test tadpoles spent the majority of their time near non-siblings than near heterospecific *S. breviceps* tadpoles (table 9, figure 4E).
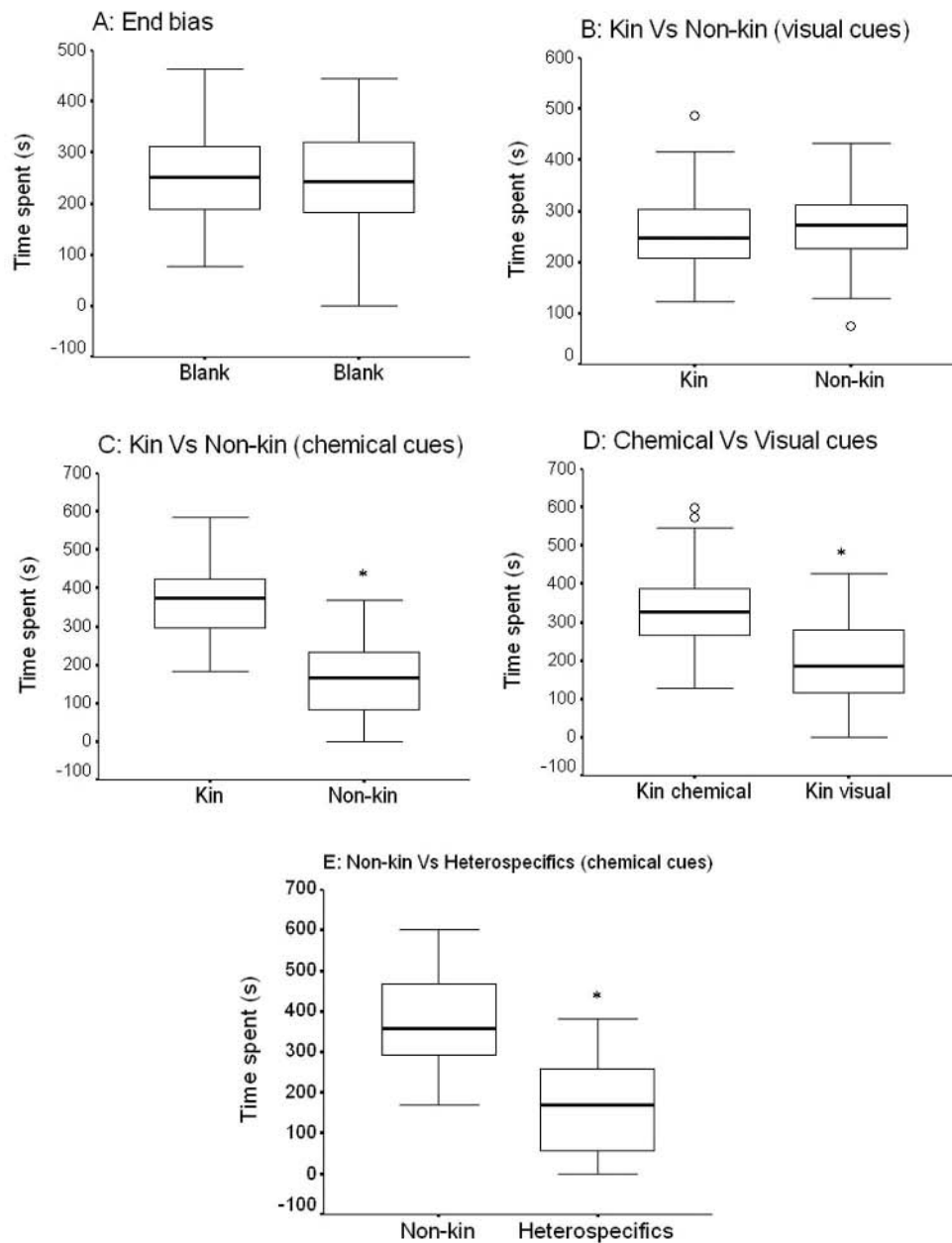
Figure 4. Box whisker plots showing the association preference of test tadpoles with respect to zones A and B. **(A)** End-bias test – both stimulus zones blank. **(B)** Tests with visual cues of kin vs non-kin. **(C)** Tests with chemical cues of kin vs chemical cues of non-kin. **(D)** Tests with chemical cues of kin vs visual cues of kin. **(E)** Tests with chemical cues of homospecific (non-kin) vs chemical cues of heterospecific (*S. breviceps*) tadpoles. Horizontal bars in the boxes represent medians, whiskers represent the farthest data points that are not outliers, open circles above the whiskers represent outliers. The asterisks denote a significant difference in time spent by test tadpoles between the two zones.

4. Discussion

Several studies have documented the kin recognition phenomenon and its possible significance in diverse groups of animals ranging from protozoa to mammals as well as plants (Waldman 1987; Saidapur and Girish 2000; Mateo and Johnston 2003; Holmes 2004; Pakkasmaa and Laurila

2004; Dudley and File 2007). Yet, the mechanisms as well as the functions of kin recognition are poorly understood. Among amphibians, kin recognition ability has mainly been documented in the larval stage (references in Waldman 1991; Saidapur and Girish 2000; Gramapurohit *et al.* 2006). Studies on *B. melanostictus* have shown that tadpoles reared in isolation from a very early embryonic

Table 6. Association choice of *B. melanostictus* tadpoles between kin and non-kin, both providing visual cues (experiment 2)

Parental line	Number spending most[a] time near visual cues of		Time (s) spent in zones[b] visual cues of (mean ± SE)	
	Kin	Non-kin	Kin	Non-kin
P1	5	5	267.50 ± 30.92	261.3 ± 28.05
P2	5	5	260.7 ± 24.88	270.2 ± 21.64
P3	3	7	245.2 ± 19.29	286.5 ± 13.59
P4	3	7	254.4 ± 33.76	260.4 ± 25.70
P5	5	5	260.8 ± 08.98*	258.5 ± 16.21

[a] Compared using binomial test. [b] Time spent in stimulus zones was compared using a random expectation by the Wilcoxon-signed rank test. *Significantly different.
Fisher test of combining probabilities used for overall results: $-2\sum \ln P = 4.28$, $\chi^2 [10]$: $P>0.05$ (number data); $-2\sum \ln P = 6.70$, $\chi^2 [10]$: $P>0.05$ (time data).

Table 7. Association choice of *B. melanostictus* tadpoles between kin and non-kin, both providing chemical cues (experiment 2)

Parental line	Number spending most[a] time near chemical cues of		Time (s) spent in zones[b] chemical cues of (mean ± SE)	
	Kin	Non-kin	Kin	Non-kin
P1	9*	1	399.90 ± 33.49*	128.4 ± 34.93
P2	7	3	299.90 ± 21.90*	221.4 ± 20.84
P3	7	3	361.80 ± 32.09*	194.70 ± 33.05
P4	9*	1	387.70 ± 32.45*	152.40 ± 27.96
P5	8	2	377.90 ± 30.89*	123.20 ± 36.45

[a] Compared using binomial test. [b] Time spent in stimulus zones was compared using a random expectation by the Wilcoxon signed-rank test. *Significantly different.
Fisher test of combining probabilities used for overall results: $-2\sum \ln P = 23.64$, $\chi^2 [10]$: $P<0.01$ (number data); $-2\sum \ln P = 21.954$, $\chi^2 [10]$: $P<0.02$ (time data)

Table 8. Association choice of *B. melanostictus* tadpoles between kin (visual cue) and kin (chemical cue) (experiment 2)

Parental line	Number spending most[a] time near		Time (s) spent in zones[b] (mean ± SE)	
	Kin (chemical cue)	Kin (visual cue)	Kin (chemical cue)	Kin (visual cue)
P1	7	3	347.80 ± 39.72*	163.40 ± 39.23
P2	8	2	316.90 ±38.97*	228.20 ± 39.39
P3	9*	1	300.40 ± 31.15*	199.80 ± 23.36
P4	8	2	340.7 ± 42.23*	172.80 ± 34.45
P5	9*	1	357.2 ± 29.87*	184.2 ± 31.32

[a] Compared using binomial test. [b] Time spent in stimulus zones was compared using a random expectation by the Wilcoxon signed-rank test. *Significantly different.
Fisher test of combining probabilities used for overall results: $-2\sum \ln P = 26.46$, $\chi^2 [10]$: $P<0.01$ (number data); $-2\sum \ln P = 42.2$, $\chi^2 [10]$: $P<0.001$ (time data).

stage (Gosner stage 12) recognize their siblings later, thereby ruling out familiarity-based kin recognition (Saidapur and Girish 2000). Instead, they indicate a genetic-based mechanism of kin recognition. Recent studies on tadpoles of *Xenopus laevis* provide evidence for a genetic basis of kin recognition by self-referent major histo-compatibility complex (MHC) matching (Villinger and Waldman 2008).

It is generally believed that anuran tadpoles possess poor vision. Therefore, varieties of functions that involve the detection process (e.g. food, predator, prey, siblings, etc.) in general may depend upon other sensory cues rather than

Table 9. Association choice of *B. melanostictus* tadpoles between non-kin and heterospecifics (*S. breviceps*) both providing chemical cues (experiment 2)

Parental line	Number spending most[a] time near chemical cues of		Time (s) spent in zones[b] chemical cues of (mean ± SE)	
	Non-kin	Heterospecifics	Non-kin	Heterospecifics
P1	8	2	350.7 ± 37.87*	196.8 ± 38.05
P2	9*	1	415.4 ± 40.40*	139.1 ± 37.80
P3	9*	1	382.00 ± 41.55*	158.90 ± 37.20
P4	8	2	387.10 ± 42.19*	156.00 ± 34.80
P5	7	3	326.80 ± 29.88*	174.4 ± 38.89

[a] Compared using binomial test. [b] Time spent in stimulus zones was compared using a random expectation by the Wilcoxon signed-rank test. *Significantly different.

Fisher test of combining probabilities used for overall results: $-2\sum \ln P = 26.52$, $\chi^2[10]$: $P<0.01$ (number data); $-2\sum \ln P = 51.82$, $\chi^2[10]$: $P<0.001$ (time data).

visual cues. In murky waters and benthic regions with low visibility coupled with poor vision, tadpoles of most anurans may have to predominantly rely upon chemical cues for detection of food, prey, predators as well as kin and non-kin (Blaustein and O'Hara 1982; Waldman 1985, 1986, 1987, 1991; Cornell *et al.* 1989; Kiseleva 1989; Blaustein *et al.* 1993; Gramapurohit *et al.* 2006). The findings of our study clearly show that *B. melanostictus* tadpoles recognize their siblings based on chemical cues and that visual cues are ineffective even in clear waters.

Regardless of the functional significance and benefits of kin association, it is clear that several species of anuran tadpoles discriminate between kin and non-kin. Yet, it is not clear whether the association of test tadpoles with kin results from an attraction towards them or due to repulsion from non-kin induced by certain signals released by the latter. Studies in this direction are limited to *Rana cascadae* (Blaustein and O'Hara 1983, 1987) and *Bufo americanus* (Waldman 1985). Blaustein and O'Hara (1983, 1987) suggest that *R. cascadae* tadpoles show a positive preference for kin rather than an avoidance of non-kin. On the other hand, Waldman (1985) showed that *B. americanus* tadpoles avoid non-siblings. The conclusions of the two studies are thus in sharp contrast to each other. In our study, we found that *B. melanostictus* tadpoles prefer to associate with non-kin rather than the blank zone in the absence of kin members, indicating they are not repelled by non-kin. Thus, despite kin recognition abilities, toad tadpoles associate with non-kin in the absence of kin. Further, when given a choice between non-kin and heterospecifics, toad tadpoles prefer to be with homospecifics. In this study, all tadpoles were reared on spinach and hence dietary cues, if any, would be shared. Therefore, recognition of kin or non-kin based exclusively on dietary cues may be ruled out. In fact, not only was kin discrimination by the toad tadpoles evident but also their preference for associating with non-kin in the absence of

kin. This may be because they share some traits vis-à-vis genes among individuals of a given species. This view is supported by the fact that when test tadpoles were exposed to non-kin and heterospecifics, they preferred to stay near non-kin. These findings also suggest that a phenotype matching mechanism may also operate in *B. melanostictus* tadpoles to discriminate between homospecific and heterospecific species.

In summary, our study shows that the association of *B. melanostictus* tadpoles with kin members is due to attraction rather than repulsion from the chemical cues of non-kin. Further, the study shows that in addition to kin discrimination, species discrimination also operates in *B. melanostictus* tadpoles. Selection may have favoured living with non-kin in the absence of kin members to possibly derive the benefits of group living, especially in tadpoles which are gregarious in nature (e.g. *B. melanostictus* tadpoles in the present study).

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