Aggregation behaviour in *Rana cascadae* tadpoles: association preferences among wild aggregations and responses to non-kin

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Abstract. In laboratory experiments, wild-caught *Rana cascadae* tadpoles preferred to associate with members of their natural aggregation over members from a second aggregation collected from the same pond. Laboratory-reared tadpoles also preferred to associate nearest to a sibling stimulus group over a stimulus group containing no tadpoles. However, when tadpoles were given a choice of associating between a stimulus group containing conspecific non-siblings and an empty compartment, there was random association. This suggests that tadpoles are gregarious with kin, but are not as likely to form aggregations composed solely of non-kin.

It is evident that individuals of numerous species representing various taxonomic groups can discriminate between kin and non-kin (e.g. reviews by Colgan 1983; Holmes & Sherman 1983; Sherman & Holmes 1985; Hepper 1986; Blaustein et al. 1987a; Porter, in press). These studies have provided important information on the ontogeny of kin recognition behaviour in particular and on the development of behaviour in general. These studies have also enhanced our knowledge of the evolution of social behaviour as it relates to the concepts of inclusive fitness and kin selection (Hamilton 1964; Maynard Smith 1964).

Studies of kin recognition have revealed important differences in how kin recognition is manifested in different species. For example, sweat bees, *Lasioglossum zephyrum*, can distinguish between individuals of varying degrees of relatedness, and familiarity with an individual is not necessary for subsequent recognition (Greenberg 1979; Buckle & Greenberg 1981). Other species of social insects use nest association from which they might learn recognition cues (e.g. paper wasps, *Polistes fuscatus*, Shellman & Gamboa 1982), and still other species can discriminate between full and half sisters that were raised in the same environment, suggesting that association learning per se is not important in recognition (e.g. honey bees, *Apis mellifera*, Getz & Smith 1983).

Certain species of small mammals can identify unfamiliar kin (e.g. *Spermophilus beldingi*, Holmes & Sherman 1982; *Acomys cahirinus*, Porter et al. 1983) whereas other species can identify only familiar individuals (*Spermophilus tridecemlineatus*, Holmes 1984). Familiarity also plays an important role in kin recognition in some bird species (e.g. bank swallows, *Riparia riparia*, Beecher & Beecher 1983) but apparently not others (e.g. Japanese quail, *Coturnix coturnix*, Bateson 1982).

Kin recognition systems also differ among anuran amphibian larvae of several species (e.g. Blaustein & O’Hara 1981, 1982a, b, 1986a; O’Hara & Blaustein 1981, 1982, 1985; Waldman 1981, 1984, 1985). A recognition system based on waterborne chemical cues (Blaustein & O’Hara 1982b), enables Cascades frog *Rana cascadae* tadpoles to distinguish between kin and non-kin with the rearing regime exerting little influence on the development of kin recognition behaviour (Blaustein & O’Hara 1981, 1983; O’Hara & Blaustein 1981, 1985). Furthermore, *R. cascadae* tadpoles can distinguish between full and half siblings (both maternal and paternal) and between half siblings (both maternal and paternal) and non-siblings (Blaustein & O’Hara 1982a). The ability to discriminate between kin and non-kin is retained after metamorphosis (Blaustein et al. 1984).

The sensitive kin recognition system displayed by *R. cascadae* tadpoles is not typical of tadpoles of other species. For example, tadpoles of *R. aurora*, a species closely related to *R. cascadae*, can discriminate between kin and non-kin only in early freeswimming larval stages (Blaustein & O’Hara 1986a). *Rana sylvatica* tadpoles also display a relatively sensitive kin recognition system in laboratory tests (Waldman 1984). However, they generally do not aggregate in nature and often avoid one another (Hassinger 1972; DeBenedictis 1974;
Waldman 1984), making it difficult to interpret the results of laboratory kin recognition tests. Tadpoles of the spotted frog, R. pretiosa and Pacific treefrog, Hyla regilla, show no ability to discriminate between kin and non-kin (O'Hara & Blaustein, unpublished data). Moreover, kin recognition in tadpoles of two toad species, Bufo boreas and B. americanus, seems to be greatly influenced by the rearing regime (Waldman 1981; O'Hara & Blaustein 1982). With the exception of R. sylvatica, the differences in the kin recognition systems of anuran amphibian larvae are generally correlated with key life history and behavioural characteristics.

In our previous experiments we assessed the discriminatory abilities of tadpoles by allowing them to choose to associate with either of two stimulus groups in the laboratory or to form groups in natural ponds in the field after they were reared under a variety of regimes in the laboratory (e.g. Blaustein & O'Hara 1981, 1982a; O'Hara & Blaustein 1981, 1985). In the present study, we assessed the affinity of tadpoles for members of their natural wild-caught aggregations. This is the first time that laboratory tests, in the context of kin recognition, have been conducted on tadpoles that hatched and developed in the field in their natural ponds. We also conducted tests to investigate whether positive attraction to kin or avoidance of non-kin is the primary means of achieving kin association. This is an important component in recognition that is often overlooked in kin recognition studies.

METHODOLOGY

Larval Behaviour and Rearing Conditions

*Rana cascadae* inhabits montane streams and lakes of the Cascade Mountain Range of Oregon, Washington, and California, and the Olympic Mountain Range of Washington (U.S.A.). *Rana cascadae* tadpoles are (1) highly social and exist in aggregations much smaller than the size of a clutch (O'Hara 1981; O'Hara & Blaustein 1981, 1982) and (2) do not disperse far from their hatching sites (O'Hara 1981). Thus, it is possible that groups of *R. cascadae* tadpoles are composed primarily of related individuals (O'Hara & Blaustein 1981, 1982, 1985; Blaustein et al. 1987a).

For experiment 1, we collected three distinct *R. cascadae* tadpole aggregations (A, B, C) in July 1985, and three aggregations (D, E, F) in July 1986 from one pond in Marion County, Oregon. For experiment 2, we collected three fertilized clutches (1, 2 and 3) of eggs from the same population in July 1985. The tadpoles and eggs were transported separately in containers that held each distinct group. Each tadpole aggregation and clutch was placed in a 38-litre aquarium with dechlorinated tap water, and kept under a 14:10 L:D photoperiod at 20–22°C. Thus, from capture, tadpoles were subjected only to members of their own group and members of each clutch were exposed only to siblings during rearing. Water was changed in all rearing aquaria every 3–5 days. The tadpoles were fed rabbit pellets and tropical fish food daily. All tadpoles were released back into their natural ponds in the Cascade range when the experiments were completed.

Apparatus

A tank (122 × 44 × 30 cm) was used to test two tadpoles simultaneously (but independently) for sibling group preferences (figures of this apparatus have been published previously; e.g. O'Hara & Blaustein 1981). To create end compartments for housing stimulus tadpoles (see below), a partition of plastic mesh of 1.5 mm was placed 15 cm from each end of the tank. A central longitudinal partition was opaque and water-tight.

Testing Procedures

Experiment 1

We tested tadpoles from wild-caught aggregations within 1 week of their capture. Prior to each test, the tank was filled to a depth of 6 cm with 34 litres of dechlorinated tap water (at room temperature). After 2–4 min, 20 tadpoles from members of one natural aggregation and 20 from a second natural aggregation were placed in opposite end compartments and left undisturbed for 15 min. These tadpoles were used as stimulus groups. Two members of one of the natural aggregations were tested simultaneously in each trial and were released at the tank centre, with one being placed on each side of the longitudinal partition. The test tadpoles were allowed to acclimate for 10 min. The time spent (s) by each tadpole in the half nearest their aggregation members or the half nearest the members of the foreign aggregation was recorded.
Table 1. Association preferences of wild-caught *Rana cascadae* tadpoles (time for each test = 1200 s)*

<table>
<thead>
<tr>
<th>Group (N)</th>
<th>No. spending most of time nearest own aggregation (binomial P)†</th>
<th>Time (s) spent on side nearest own aggregation (mean ± SEM)</th>
<th>Wilcoxon T</th>
<th>Wilcoxon P</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (15)</td>
<td>11 (&gt;0.05)</td>
<td>653.0 (±20.6)</td>
<td>21</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>B (20)</td>
<td>15 (&lt;0.042)</td>
<td>681.8 (±20.8)</td>
<td>22</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>C (19)</td>
<td>17 (&lt;0.004)</td>
<td>658.0 (±17.5)</td>
<td>20</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>D (18)</td>
<td>13 (&gt;0.05)</td>
<td>646.7 (±19.6)</td>
<td>40</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>E (16)</td>
<td>13 (&lt;0.02)</td>
<td>670.1 (±31.2)</td>
<td>27</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>F (15)</td>
<td>12 (&lt;0.04)</td>
<td>666.1 (±24.1)</td>
<td>21.5</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

* Tadpoles were tested at Gosner (1960) stages 37–41.
† Number tested (sample size determined by the number of tadpoles available).
‡ Binomial for all samples combined: P < 0.0001.

for four 5-min trials at 10-min intervals. Observations were taken from behind a plastic blind. Following each test, the tank was drained and rinsed thoroughly. Tadpoles from aggregation B were used as the alternative stimulus when tadpoles from aggregations A and C were tested. Tadpoles from aggregation E were used as the alternative stimulus when tadpoles from groups D and F were tested. Tadpoles from groups C and D were used as alternative stimuli when tadpoles from groups B and E were tested, respectively. No tadpole was tested more than once and the same stimulus animals were used in no more than 10 tests. We alternated stimulus groups from one end to the other between tests. Other controls for the apparatus and testing procedures have been reported elsewhere (e.g., Blaustein & O’Hara 1981; O’Hara & Blaustein 1981).

Experiment 2
We used tadpoles from clutches 1 and 2 to test whether sibling recognition in *R. cascadae* tadpoles is based on attraction to kin or on aversion to non-kin (see Blaustein & O’Hara 1983). Tadpoles from clutch 3 were used solely as stimulus animals. The procedures we used were similar to those described in experiment 1. In one series of tests, tadpoles were given a choice of associating near a stimulus compartment containing members of their own sibling group or near a stimulus compartment containing no tadpoles. In a second series of tests, tadpoles were given a choice of associating near a stimulus end containing unrelated conspecifics or near an end containing no tadpoles. In these tests all stimulus tadpoles and test individuals were matched for body size and developmental stage (Gosner 1960). Test tadpoles were used in only one test and stimulus tadpoles were used in no more than six tests. The stimulus compartments that contained tadpoles had 25 individuals. All other testing procedures and time measurements were identical to those described in experiment 1.

Statistical Tests
We used the total amount of time spent on sibling and non-sibling halves of the test tank as a measure of a tadpole’s association preference. The Wilcoxon signed-ranks test was used to test differences in time spent on one side (towards tadpoles from their own aggregation, siblings, or non-siblings depending on the experiment) from a hypothetical random time (600 s) expected under
Table II. Association preferences of *Rana cascadae* tadpoles reared with siblings and given a choice of associating with siblings (S) and an empty stimulus compartment (E) or non-siblings (Non-S) and an empty stimulus compartment (E)

<table>
<thead>
<tr>
<th>Sibling group</th>
<th>Age in days (Gosner stage)</th>
<th>No. spending most of time nearest:</th>
<th>Binomial P</th>
<th>Time (s) spent nearest S/Non-S (mean ± se)</th>
<th>Wilcoxon T(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stimulus group: siblings</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>29–32</td>
<td>15</td>
<td>5</td>
<td>0.042</td>
<td>652.8 (± 17.2)</td>
</tr>
<tr>
<td>N = 20</td>
<td>(31–33)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>27–29</td>
<td>15</td>
<td>5</td>
<td>0.042</td>
<td>697.2 (± 27.9)</td>
</tr>
<tr>
<td>N = 20</td>
<td>(31–33)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stimulus group: non-siblings</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>34–37</td>
<td>11</td>
<td>8</td>
<td>0.50</td>
<td>609.7 (± 33.4)</td>
</tr>
<tr>
<td>N = 20*</td>
<td>(34–37)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>27–29</td>
<td>13</td>
<td>7</td>
<td>0.26</td>
<td>612.8 (± 17.4)</td>
</tr>
<tr>
<td>N = 20</td>
<td>(31–33)</td>
<td></td>
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</tbody>
</table>

* One tadpole spent an equal amount of time on both sides of the test tank.
† NS = Not significant (P > 0.05).

the null hypothesis. The number of tadpoles spending most of their time on either side of the test tank was compared using the binomial test. All tests were two-tailed.

**RESULTS**

**Experiment 1**

Tadpoles spent most of their time on the side of the test tank nearest the stimulus group that housed members of the test animal's natural aggregation. Test tadpoles swam from end to end in trials, and most activity and resting took place within 10 cm of the screen of the end compartments. All six groups that were tested displayed a non-random distribution of time within the test tank (Table I). The number of tadpoles that spent most of their time nearest this portion of the test tank was significantly different from random in four of the six groups.

**Experiment 2**

*Rana cascadae* tadpoles preferred to associate nearest to the stimulus side of the test tank containing members of their sibling group over the side containing no tadpoles (Table II). Both the mean time spent on the sibling side of the test tank and the number of tadpoles spending most of their time in this portion of the tank were significantly different from random. When given a choice of associating nearest unrelated conspecifics or nearest the stimulus side containing no tadpoles, *R. cascadae* tadpoles displayed a random distribution within the test tank. These results indicate that *R. cascadae* tadpoles are positively attracted to siblings (Table II).

**DISCUSSION**

*Rana cascadae* tadpoles preferred to associate with members of their natural aggregation over members of a second aggregation from the same pond. Because the genetic affinity of the tadpoles within groups was not known, these were only indirect tests of 'kin recognition' behaviour. However, the test tadpoles hatched in the field and were free-swimming for at least several weeks and exhibited a tendency to associate with tadpoles that were at least 'familiar' to them. Based on the larval characteristics of low dispersal from oviposition sites and high affinity to aggregate, these results suggest that the tadpoles were responding preferentially to putative kin.

Several mechanisms have been proposed to explain how an individual actually discriminates between kin and non-kin and in some cases between close relatives and distant ones (for recent
discussions of these mechanisms see Beecher 1982; Blaustein 1983; Holmes & Sherman 1983; Sherman & Holmes 1985; Blaustein et al. 1987a). It is becoming clear that the mechanism of familiarity, in which relatives learn to recognize familiar individuals, is the most common mechanism employed (see detailed discussion in Bekoff 1981). Familiarity plays at least some role in the recognition abilities of the larvae of two toad (Bufo boreas and B. americanus) and two frog (Rana aurora and R. sylvatica) species (Waldman 1981, 1984; O'Hara & Blaustein 1982; Blaustein & O’Hara 1986a). When using the mechanism of familiarity, an individual may develop a recognition template by learning the odour or some other phenotypic marker of animals it was reared with. Although R. cascadae tadpoles do not rely on familiarity with other tadpoles as the main mechanism of kin recognition (Blaustein & O’Hara 1981; Blaustein 1983; Blaustein et al. 1987a, b), familiarity may be used in conjunction with another mechanism or in situations in which there are no ambiguities that could hamper clear discrimination between kin and non-kin (Bekoff 1981; Blaustein et al. 1987b). For example, familiarity would not be an efficient mechanism for discriminating between kin and non-kin if numerous non-kin develop near or interact with related individuals (see Bekoff 1981; Beecher 1982; Holmes & Sherman 1982; Blaustein et al. 1987b for discussions).

An important component of recognition mechanisms, about which we know very little, is whether positive attraction to kin or avoidance of non-kin is the primary means of achieving kin association. The results of experiment 2 suggest that R. cascadae tadpoles preferred to associate nearest the stimulus portion of the test tank containing siblings over the portion of the test tank containing no tadpoles. This simply corroborates our earlier findings that R. cascadae tadpoles are attracted to siblings. However, R. cascadae test tadpoles displayed a random association of times within the test tank when they were given a choice of associating nearest a stimulus group composed of unrelated conspecifics and an empty compartment. These results support our previous findings suggesting that R. cascadae tadpoles are not repelled by non-siblings (Blaustein & O’Hara 1983). In those tests, tadpoles avoided a neutral middle portion of the test tank and spent most of their time within the third of the tank nearest siblings. The time tadpoles spent in the third of the test tank nearest non-siblings was closer to a random expectation. These results are in contrast to those found by Waldman (1985) for B. americanus tadpoles. In that study, B. americanus tadpoles failed to distinguish between sibling-conditioned water and blank water. However, when allowed to choose between non-sibling-conditioned water and blank water, the latter was preferred. Although the effect of tadpole presence (i.e. visual cues) on the response was not tested, these results suggest that avoidance of non-siblings rather than positive attraction to siblings is the basis of kin association in B. americanus tadpoles.

The finding that R. cascadae tadpoles did not prefer to associate with unrelated conspecifics contrasts somewhat with results obtained previously by O’Hara (1981). O’Hara (1981) found that when R. cascadae tadpoles were given a choice of associating near conspecifics or away from conspecifics in a laboratory test tank, they chose to associate close to them. However, in O’Hara’s (1981) study, tadpoles were allowed to use only visual cues in association tests, whereas in the present study, tadpoles could use water-borne chemical cues as well as visual cues. Water-borne chemical cues are the basis for discriminating between kin and non-kin in R. cascadae tadpoles (Blaustein & O’Hara 1982b) and they probably played an important role in influencing the results of our present experiment. Visual cues may allow tadpoles to orient towards conspecifics and chemical cues are probably used for the more fine-tuned process of discriminating between kin and non-kin (Blaustein & O’Hara 1982b). However, additional experiments using laboratory techniques similar to those described in this paper (O’Hara, unpublished data) have shown that tadpoles of R. cascadae associate preferentially with members of their own species (groups composed primarily of non-siblings) over tadpoles of a different species (B. boreas and H. regilla). In the context of our present findings that R. cascadae tadpoles are neither attracted nor repelled by non-siblings, the mechanism used to associate with conspecifics would seem to involve avoidance of heterospecifics. Further tests are needed, however, to substantiate this hypothesis.

The overall implication of our results is that groups composed primarily of kin will form readily, but groups composed primarily of non-kin are not likely to form. Previously (Blaustein & O’Hara 1983), we have shown that R. cascadae tadpoles failed to associate preferentially with a stimulus
group composed of 25% siblings and 75% non-siblings over a pure non-sibling group. However, tadpoles did associate preferentially with a stimulus group composed of 50% siblings and 50% non-siblings over a pure non-sibling group. These findings, along with our present results, indicate that R. cascadae tadpoles will form groups only if there is a threshold number of related individuals within the group. R. cascadae tadpoles may be highly social towards kin and may be a-social towards non-kin.

By showing that R. cascadae tadpoles display an affinity for members of their natural aggregation, we have corroborated our earlier field experiments illustrating that in nature, tadpoles sort into groups composed primarily of kin (O'Hara & Blaustein 1985). Field observations of R. cascadae tadpoles and controlled field and laboratory experiments using R. cascadae larvae are consistent with a kin selection model (Hamilton 1964; Maynard Smith 1964). Rana cascadae larvae are almost never found alone in nature but are in close, social aggregations composed generally of fewer than 100 individuals, a number much smaller than their clutch size (O'Hara 1981; Blaustein & O'Hara 1986b). Field experiments conducted by O'Hara (1981) illustrated that dispersal from sites of oviposition is low. Consequently, tadpoles may have numerous opportunities to interact with kin. Rana cascadae tadpoles are subjected to intense predation pressure and they show an alarm response from chemicals emanating from damaged conspecifics (Hews & Blaustein 1985). The alarm response could be important in warning relatives within an aggregation. Kin recognition could enhance the ability of related individuals to reform groups after disturbances, such as those caused by predation. Altruists in groups with many relatives could increase their inclusive fitness by aiding (such as warning) members of the group, compared to those individuals giving similar aid in groups with fewer or no kin.

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