

# Pesticide Effects on Kin Selection in Amphibians

G.E. Rappi and K.B. Sullivan

*Department of Biological Sciences, College of Liberal Arts and Sciences*

## 1. Introduction

Recent studies have indicated that environmentally realistic concentrations of pesticides may alter behaviors of amphibian and fish species impacting larval survivorship and growth. Alterations in fish swimming have been reported in response to chronic and acute exposure to atrazine [1]. Tadpoles of *Bufo woodhousii* exposed to atrazine exhibited significantly reduced activity [2]. Swimming activity in the African clawed frog has been reported to be decreased in the presence of atrazine [3]. Sub-lethal exposure to the organophosphate insecticide carbaryl significantly decreased activity and proportion of time spent foraging in *Hyla versicolor* [4]. While anti-predator and foraging behaviors are necessary for tadpole survival, kin recognition is in many amphibian species an equally important behavior that promotes aggregation and may also reduce predation risk. Kin recognition (in tadpoles mediated by water-borne chemical cues [5]) is the ability of an individual to associate with relatives in preference to non-relatives. Aggregation of kin may serve as an effective anti-predator defense and may also improve swimming, feeding efficiency and thermoregulation among related individuals. It has been reported that *B. americanus*, a closely related species to *B. woodhousii*, exhibits kin recognition behaviors.

The purpose of this study was to determine the effect of two pesticides, the triazine herbicide atrazine and the organophosphate insecticide chlorpyrifos, on kin recognition and activity of tadpoles of *B. woodhousii*. During peak application periods of atrazine during the spring, concentrations of 250 µg/L or greater have been reported in some Kansas streams. In bodies of water adjacent to cornfields, chlorpyrifos has been detected at levels up to 67 µg/L [6]. The concentrations of atrazine (0, 20, and 200 µg/L) and chlorpyrifos (0, 1 and 10 µg/L) used in this experiment are therefore possibly encountered by amphibians that breed in temporary pools near agricultural fields during spring reproductive activity. The null hypothesis was that preference for kin and activity were the same among control and pesticide exposed animals.

## 2. Materials and Methods

Two pairs of adult toads were collected from different breeding ponds to assure that both related and unrelated tadpoles would be available for testing. Pairs were induced to breed with human chorionic gonadotropin and housed in breeding tanks with moderately hard water (MHW). Fertilized eggs were maintained in breeding tanks for 5 days. For each clutch, 20 randomly chosen tadpoles ('receivers') were assigned to each of 12 different aquaria and exposed to atrazine (0, 20, 200 µg/L) or chlorpyrifos (0, 1, 10 µg/L) in 6 L of MHW for two weeks (Gosner stages 29-40). Tanks were aerated and complete water change and chemical renewal was made every 3 or 4 days. Tadpoles were fed ad libitum a crushed mix of algae disc and commercial frog pellets.

Tadpoles (n=200) not chosen as receivers, termed the 'cue' animals, were maintained in MHW in large tanks for separate clutches and fed in a similar manner. A plastic tank with an internal compartmentalized plexiglass cage (69 x 38 x 10 cm) was used to test two tadpoles simultaneously for sibling group preferences. Each end compartment (9 cm) was separated by a 50 cm central area which was subdivided for each receiver tadpole being tested. The bottom of the central department was marked to distinguish three areas: a middle zone and two zones adjacent to each cue end. MHW without chemical was added to a depth of 6 cm. This experimental approach follows that used in a number of studies designed to examine kin selection in amphibians [e.g., 7]. Twenty cue tadpoles from each of the two clutches were placed in opposite end compartments. After a 10 minute acclimation, receiver tadpoles were individually placed in the central compartments and acclimated for 10 minutes; tadpoles were then videotaped for 10 minutes. Total time actively moving and time spent in each area near kin (Ktime) or non-kin (Nktime) tadpoles were recorded for each receiver. Chambers were cleaned between each test event and clutches were switched to opposite end compartments. Two-way ANOVA was used to determine the effects of clutch and chemical treatments for both the atrazine and chlorpyrifos experiments.

### 3. Results and Discussion

#### Atrazine Experiment

ANOVA results (Table 1) indicated significant differences among clutches, among atrazine concentrations and treatment interaction for mean total time active during the 10 minute observation. Tadpoles in both clutches exposed to 200 µg/L atrazine exhibited significantly less activity than control animals. Tadpoles from clutch 2 exhibited greater mean activity, in general, than tadpoles from clutch 1, indicating inter-clutch variation for this trait. The significant interaction between clutch and atrazine treatment reflects the tendency exhibited by clutch 1 animals to decrease activity at both 20 and 200 µg/L exposures, while clutch 2 animals tended not to decrease activity until 200 µg/L.

Although only significant at  $p = 0.076$  (Table 1), tadpoles exhibited a tendency to spend less time in the kin end of the tank with increasing exposure to atrazine. No differences were noted in mean time spent near non-kin individuals.

#### Chlorpyrifos Experiment

ANOVA results (Table 2) indicated a non-significant effect of chlorpyrifos on mean activity, but a near significant effect on both time spent near non-kin and kin ends of the experimental tank. There was, however, no obvious dose-related effect. Tadpoles exposed to 1 mg/L chlorpyrifos tended to spend more time near kin and less time near non-related individuals than tadpoles in either the 0 or 10 µg/L treatments.

Although chlorpyrifos did not impact mean activity time, there were significant differences in activity among the clutches (Table 2), again illustrating inter-clutch variability in behavior as observed in the atrazine experiment. Clutch 2 tadpoles exhibited significantly greater activity levels in all chlorpyrifos treatments.

### 4. Conclusions

Tadpoles of *B. woodhousii* exhibited significantly reduced levels of activity after exposure to 200 µg/L atrazine. Similar concentrations may be found in temporary breeding locations adjacent to agricultural fields. Previous studies in our laboratory have indicated that atrazine is associated with delayed metamorphosis and growth in *X. laevis*. Reductions in activity are predicted to be associated with reductions in foraging activity and growth, which may in turn provide an explanation for delayed metamorphosis. Predator escape in tadpoles is often accomplished by short bursts of activity interrupted by periods of inactivity under cover. A reduction in activity levels may increase vulnerability to predation.

Tadpoles exposed to atrazine also exhibited a near significant tendency for decreased amounts of time spent near kin, suggesting an alteration in kin selection behavior that may negatively impact survivorship by disruption of aggregative behavior. Chlorpyrifos exposure did not significantly affect activity of tadpoles; tadpoles reared in 1 µg/L chlorpyrifos spent less time near kin and more time near non-kin animals in the other treatment groups. These results provide a strong impetus to continue an examination of pesticide effects on kin selection behavior. This study also indicates that intraspecific variations in behavioral responses to pesticides are likely, which should be considered when designing experiments to detect subtle, yet ecologically relevant, behavioral endpoints.

### 5. References

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Table 1. Results of 2 X 2 ANOVA for tadpoles exposed to atrazine. Values given are F statistics and p values. Degrees of freedom are given in parentheses.

Trait	Clutch (1,114)	Atrazine (2,114)	Clutch x Atrazine (2,114)
Active (s)	10.59, <0.001	10.20, < 0.001	9.06, <0.001
Ktime (s)	3.60, 0.060	2.64, 0.076	0.54, 0.584
Nktime (s)	1.97, 0.163	1.28, 0.283	0.50, 0.608

Table 2. Results of 2 X 2 ANOVA for tadpoles exposed to chlorpyrifos. Values given are F statistics and p values. Degrees of freedom are given in parentheses.

Trait	Clutch (1,114)	Chlorpyrifos (2,114)	Clutch x Chlorpyrifos (2,114)
Active (s)	50.35, <0.001	0.20, 0.822	2.29, 0.106
Ktime (s)	0.25, 0.612	2.80, 0.065	1.18, 0.3121
Nktime (s)	0.12, 0.730	2.90, 0.054	1.43, 0.243