CHEMICALLY MEDIATED MATE RECOGNITION IN THE TAILED FROG (*ASCAPHUS TRUEI*)

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1. INTRODUCTION

Amphibian communication methods are a popular field of study (Duellman and Trueb, 1994; Houck, 1998). Acoustic signals are used by the majority of adult anuran species to attract mates (Duellman and Trueb, 1994), and acoustic communication has been overwhelmingly documented in anurans. Few anurans have been reported to use visual signals for mate recognition (Summers et al., 1999). Chemical communication related to mate recognition in adult anurans has only been documented recently (Forester and Thompson, 1998; Wabnitz et al., 1999; Pearl et al., 2000), despite the evidence that chemical signals are widespread in urodeles (Houck, 1998). These alternative forms of communication can be important, especially under conditions where acoustic communication is not favored, such as in a noisy environment or in a habitat where auditorily oriented predators are abundant. The behavioral functions of non-acoustic communication in anurans are poorly understood and its ecological significance is difficult to assess due to the small number of studies available. Hence, studies of non-acoustic communication systems in anurans are of particular interest and will shed light on how different communication systems evolve under different environmental conditions.

Tailed frogs (*Ascaphus truei*) are usually found near permanent, fast flowing streams in forested areas (Metter, 1967). Because their normal habitat is usually noisy, selection may not favor the use of acoustic communication. Several morphological features of tailed frogs make it unlikely that vocal signals are used to attract mates. Tailed frogs have small lungs and lack vocal cords, a tympanic membrane, and columella, so their ability to produce and detect sound is probably poor (Noble and Putnam, 1931; Schmidt, 1970). Although other "earless" frogs do vocalize (Hetherington and Lindquist, 1999), there are no reports of any sounds produced by tailed frogs.

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As a silent, nocturnal animal, the tailed frog does not have many options available for mate attraction and recognition beyond chemical signals. Many studies have demonstrated the ability of anurans to recognize predators (Feminella and Hawkins, 1994; Flowers and Graves, 1997) and conspecifics (Graves et al., 1993). Olfaction has also been shown to play a role in spatial orientation, homing, and food location in anurans (Grubb, 1976). Studies of kin recognition in the larvae of several anuran species have shown that information about kin is most likely transmitted as chemical signals (Waldman, 1985; Blaustein et al., 1993). Kin recognition using chemical signals may continue after metamorphosis, as in *Rana cascadae* and *Rana sylvatica* (Blaustein et al., 1984; Cornell et al., 1989). Feminella and Hawkins (1994) showed that tailed frog tadpoles use chemical cues to detect predators, but no other information about chemical communication is available, especially in adults.

Tailed frogs are assumed to search for mates by crawling along the stream bottom (Jameson, 1955). If so, visual communication is unlikely, except at very short distances. Tailed frogs may simply grab onto the nearest moving object of appropriate size, but this could be costly if a predator is encountered or clasped by mistake. If a male or an unreceptive female is clasped, the ensuing wrestling match is a waste of energy (Wells, 1977) and may subject the pair to the risk of predation. A cue to guide tailed frogs toward a suitable mate would reduce the costs involved and increase the likelihood of successfully mating. Under this scenario, the ability to recognize a chemical cue would be advantageous.

The purpose of this study was to investigate the mate recognition ability of tailed frogs. Specifically, we tested the hypotheses that tailed frogs do not use visual signals to attract mates and that tailed frogs can detect waterborne chemical cues from conspecifics. We used two experiments to achieve this goal. The first experiment tested the visual mate recognition ability of males. The second experiment examined the ability of both sexes to detect waterborne chemical signals from conspecifics.

2. METHODS

2.1 Animal Collection and Care

Study animals were collected from Cold Creek, Kittitas County, Washington (T22N, R11E, S29, NW, elevation ~1000 m) on June 26, July 27, and September 22, 1998 by searching the stream banks at night. A total of 59 males and 36 females were captured. Frogs were housed separately in plastic boxes (33 cm x 20 cm x 10 cm) with gravel, water and a plastic cup for shelter and kept in a cold room (10-12 °C) on a 14h:10h L:D light cycle. They were fed 2-3 crickets (*Acheta domestica*) per week and given fresh water every other week.

2.2 Reproductive Status

Frogs used for the tests were reproductively mature to ensure the presence of any signals linked to sexual readiness. Males were considered mature if they developed black nuptial tubercles on the palm, forearm, shoulders (ventral surface), and chin (Daugherty and Sheldon, 1982a). Females were considered mature if eggs were visible through their abdominal wall or they were at least 40 mm in total length at the time of testing (Bull and Carter, 1996). Tests were performed between September 9, 1998 and November 6, 1998.

2.3 Visual Signals

We first tested the ability of males to distinguish between male and female conspecifics and between gravid or non-gravid females using visual cues only. We designed a two-way choice test apparatus for visual preference. The test apparatus was a 38 L aquarium that was divided into three chambers by glass partitions 10 cm from each end. These two stimulus chambers isolated the test frogs in the central test chamber from any chemical signals from the stimulus frogs in the stimulus chambers but could still receive visual signals. Stimulus frogs were size-matched for total length to the nearest millimeter to prevent any choices based on size. Tailed frogs are sexually dimorphic in size, so we had few male frogs large enough to pair with females. Therefore, stimulus frogs were used in five trials and were placed in opposite sides of the apparatus between trials. Damp paper towels were placed on each side of the test chamber. Test frogs were placed in the middle of the chamber under a circular glass dish (diameter 10 cm) and allowed to acclimate for five minutes. The dish was then removed and the frogs were videotaped for 10 minutes. Twenty different frogs were tested in each experiment and each frog was used only once. The damp paper towels were replaced, and the test chamber was wiped with wet paper towels between trials.

A central dividing line was drawn in the test chamber. We define preference based on the location of the head of a test frog on either side of this line. Data were collected from the videotapes and analyzed for time spent in the section of the test chamber adjacent to either stimulus frog. The videotapes were also analyzed for the first stimulus area chosen and the frequency that test frogs were observed in each stimulus area during the entire 10 minute session. The duration data were analyzed using the Wilcoxon matched pairs signed ranks test. The frequency data were analyzed with a sign test. The first choice data were analyzed with a binomial test. In one of these experiments, one frog did not make a choice for over 3 minutes, so that trial was not used in the first choice analysis.

2.4 Chemical Signals

In this experiment, we built an apparatus for a three-way choice test. The three treatments were male-conditioned water, female-conditioned water and control water. Each water treatment was conditioned by placing two reproductively mature frogs in 19 L of water for 24 hours. Control water was prepared at the same time and placed under the same conditions for 24 hours.

The experimental apparatus consisted of a test chamber constructed from a 19 L bucket attached to three other 19 L buckets. Water flowed into the chamber through three evenly-spaced holes in the sides of the bucket and out through a hole in the center of the test chamber. Treated water entered the chamber by gravity flow from the other 19 L buckets connected to the chamber with Tygon® tubing. In-line flow meters and clamps were used to keep water flowing in each tube at a rate of approximately 2 L per hour. Food coloring was placed in each treatment of signal water to make the flow more obvious to the observer. The color was rotated between each set of trials to avoid any preferences based on the food coloring. Each test subject was exposed to the three different water samples in each trial.

The frogs were videotaped for 15 minutes after a 5 minute acclimation period. The videotapes were analyzed for frequency and duration of behaviors using an ethogram. The behaviors were jump, climb, walk, and sit. Jumping is an instantaneous event, so time was not recorded for this behavior. Duration data were analyzed using the Friedman two-way analysis of variance by ranks. The frequency data were analyzed using a χ^2 test. All data were analyzed based on response frequency or time for treatment type (male, female, control) and for behavioral pattern (jump, climb walk, sit). Duration data with significant results ($P<0.05$) were further analyzed using *post hoc* comparisons to identify which treatments were different (Daniel, 1990).

3. RESULTS

3.1 Visual Signals

Twenty trials were performed in each experiment (Experiment 1: male vs. female; Experiment 2: gravid vs. non-gravid). There was no significant difference in the mean time spent in either stimulus area for the test males (Wilcoxon matched pairs signed ranks test: Experiment 1: T=1.122, P=0.262; Experiment 2: T=0.934, P=0.350). Also, no significant differences were found for the frequency observed in either side of the test chamber for the entire 10 minute period or for first choice by test males.

3.2 Chemical Signals

In terms of response frequency, males were observed significantly more often in the female stimulus area than the other two stimulus areas (Figure 1, $\chi^2_2=8.03$, n=53, P=0.018). Also, males jumped while in the female stimulus area significantly more frequently than the other two stimulus areas (Figure 1, $\chi^2_2=6.47$, n=53, P=0.039). All other behaviors by males were without significant differences.

For female test subjects, jump was observed more frequently in the male stimulus area than the other two stimulus areas (Figure 2, $\chi^2_2=17.45$, n=29, P=0.0002). No significant differences were detected in the frequency of other behavioral responses.

For duration of response, males sat in the female stimulus area significantly more than the other two stimulus areas (Figure 3, W=7.283, n=53, P=0.026). Multiple comparison tests showed that the difference existed between female and male ($P<0.05$), but not between the other treatments. No significant differences were found in other behavioral patterns for male test subjects. There were no significant differences in the duration of any of the behaviors performed by females.

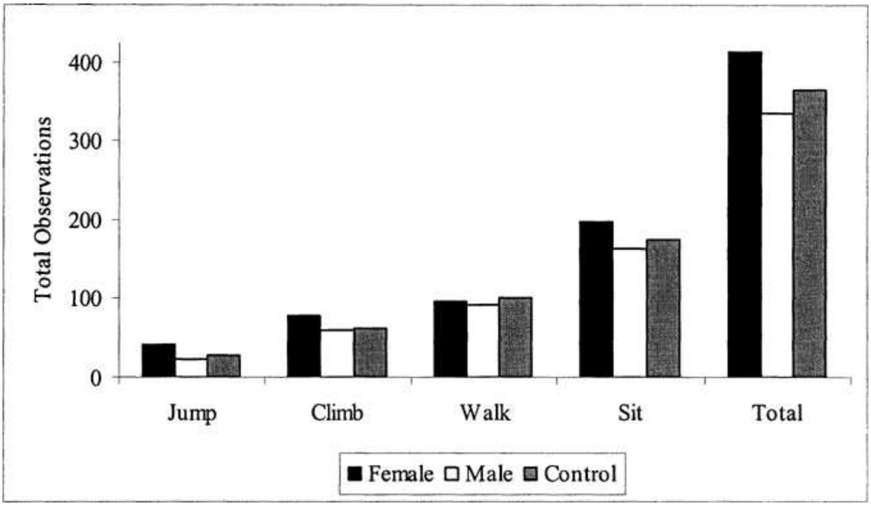

Figure 1. Total number of times all males were observed performing individual behaviors in each treatment area (n=53).

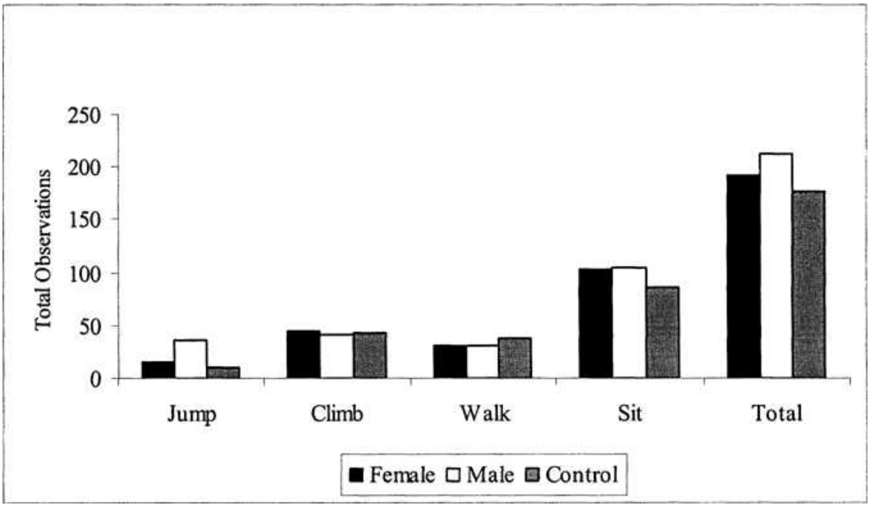

Figure 2. Total number of times all females were observed performing individual behaviors in each treatment area (n=29).

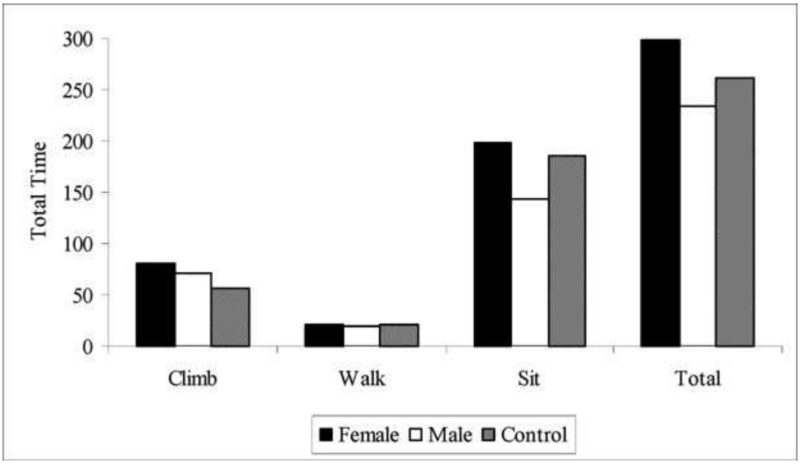

Figure 3. Total time all males were observed performing each behavior in individual treatment areas (n=53).

4. DISCUSSION

Recognition using chemical signals has been extensively demonstrated in a variety of animals, such as mammals, reptiles, amphibians, fish, and insects. In amphibians, it has been documented in caecilians (Warbeck et al., 1996) and salamanders (Houck, 1998). Attempts to study chemical recognition in anurans are relatively rare. To our knowledge, only three studies have investigated the possibility of mate recognition via chemical signals (Forester and Thompson, 1998; Wabnitz et al., 1999; Pearl et al., 2000). Thus, our study is among the first to methodologically investigate chemical signals as a mechanism of mate attraction or recognition in anurans.

No evidence from our study suggests that tailed frogs use visual cues to find mates. This is understandable because tailed frogs are nocturnal and are rarely found outside of a stream in the daytime (Metter, 1967), so visual signals would not be useful. Metter (1964) observed that males will attempt amplexus with the nearest available frog when placed in a mixed sex group of conspecifics. Many male anurans, even some without advertisement calls, use release calls when grasped by a male (Duellman and Trueb, 1994; Marco et al., 1998), but this is not the case with tailed frogs because they cannot produce sound. It seems that male tailed frogs can determine the suitability of potential mates after achieving amplexus. Wernz (1969) reported several males clasping non-gravid females and then releasing them after 20 minutes, presumably because they detected the reproductive status of the females. Duellman and Trueb (1994) suggest that continuation of amplexus by tailed frogs occurs based on the greater girth and firmness of gravid females. This method of mate recognition using tactile information may work, but only after amplexus is achieved. It would save a significant amount of time and energy, as well as reduce predation risk, if there is a pre-amplectic mechanism of recognizing suitable mates. Without using visual and acoustic signals for mate recognition, chemical communication seems to be the most likely alternative for tailed frogs.

Jameson (1955) states that tailed frogs find mates by swimming or crawling on the bottom of streams. Although this appears to be an assumption, no study has so far provided evidence to contradict these statements. If this assumption is true, then it would be advantageous to have the ability to detect a signal in the water from potential mates. Two results in our study support the presence of a waterborne mate attraction signal. One is that males and females jumped more frequently toward signals from the opposite sex than toward the other stimuli. The other is that males were recorded significantly more frequently in the female stimulus area than the other two stimulus areas. Because our design eliminated other possibilities, the signal for mate attraction or recognition is most likely a waterborne chemical signal.

Tailed frogs have a small home range (Daugherty and Sheldon, 1982b) and females sometimes aggregate (Brown, 1975), so large quantities of feces may be deposited in the same area. Feces may provide a cue for amphibians to find a mate. For instance, plethodontid salamanders recognize chemical cues in fecal pellets (Jaeger et al., 1986; Horne and Jaeger, 1988). Tailed frogs may use a similar system. Chemical signals are excellent for communicating information over short distances and the water/air interface is especially favorable for the transmission of these cues. Substances reach much higher concentrations at this interface than in air or water alone (Doving et al., 1993). Because tailed frogs are mainly aquatic, chemical signals may be particularly favored for communication.

In conclusion, we have found that tailed frogs probably do not rely on visual cues alone for mate recognition but are able to use waterborne chemical signals to find mates. While our major goal in this study was to determine whether tailed frogs are able to use chemical signals for mate recognition, the behavioral and ecological functions of mate recognition using chemical signals need to be further determined in their natural settings.

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