Oviposition Site Selection by the Túngara Frog (*Physalaemus pustulosus*)

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Oviposition behavior of *Physalaemus pustulosus* was studied using paired choice tests in small terraria. Given two possible nest construction sites (a dish with a conspecific nest in water and a dish with just water), pairs preferred to build nests in dishes that held only water. Avoidance of conspecific nests during oviposition may increase fitness by limiting potential intraspecific resource competition and by preventing tadpole growth inhibition.

Se estudió el comportamiento reproductivo de *Physalaemus pustulosus* empleando pruebas de selección de doble opción en pequeños terrarios. Dados dos sitios posibles de construcción de nidos (un recipiente con un nido coespecífico en agua y otro solo con agua) las parejas prefirieron oviponer en aquellos que contenían solo agua. Evitar los nidos coespecíficos durante la oviposición aumentaría la supervivencia, limitando la competencia intraespecífica potencial por los recursos e impediendo la inhibición del crecimiento de los renacuajos.

OVIPOSITION site selection, a potential factor affecting the reproductive behavior of anuran amphibians, has received relatively little attention. Choice of suitable sites for offspring development should strongly affect parental fitness because it can directly influence offspring survival. Many characteristics of the local environment can facilitate frog assessment of nesting site quality. Gascon and Planas (1986) suggested that *Rana sylvatica* chose nesting sites based on chemical cues such as acidity and total organic carbon. Water temperature strongly affected site selection in *R. sylvatica* (Seale, 1982; Howard, 1980; Waldman, 1982) and in other ranid species (*Rana catesbiana*: Howard, 1978; *Rana sphenocephala*: Caldwell, 1986). Additionally, anurans may assess vegetation structure (*Rana catesbiana*: Howard, 1978; *Rana clamitans*: Wells, 1977) and water depth (*Hyla pseudopuma*: Crump, 1991) of potential nesting sites. The value of a potential nesting site may also depend on biotic factors, including the presence of predators and conspecifics. Howard (1980) found that *R. sylvatica* females preferred sites with conspecific nests to facilitate communal egg deposition. Conversely, *Hyla pseudopuma* (Crump, 1991) and *Hyla chrysoscelis* (Resetarits and Wilbur, 1989) avoided conspecifics during egg deposition. *Hyla chrysoscelis* females also detected and avoided the larvae of heterospecifics that feed on *H. chrysoscelis* eggs and tadpoles as adults (Resetarits and Wilbur, 1989).

Túngara Frogs (*Physalaemus pustulosus*) have been the subjects of extensive studies in sexual selection (Ryan, 1985; Ryan and Rand, 1993, 1995). Differential female phonotaxis to a variety of male calls, both natural and synthesized, has been used to develop models of female choice and its interaction with male and female fitness (Ryan, 1985). However, much less is known about what happens once a female has chosen a mate (but for field observations of pairs in amplexus, see Ryan, 1985). Túngara frogs breed throughout the year if suitable nesting sites are available (Rand, 1983). Any place where small puddles of still water form can provide a suitable nesting site. Sites range from potholes, tapir tracks, and large leaves to human-made ditches, road ruts, and even plastic bowls (Rand, 1983; Ryan, 1985).

It is unclear whether amplexed females assess the quality of nesting sites or whether other characteristics might be used to discriminate among the plethora of sites available to them. Sexton and Ortleb (1966) suggested that females preferred to construct nests against vertical surfaces touching the water and in shallow water. Tárano (1998) found that females used visual cues to nest preferentially in covered sites. Pairs constructed nests under artificial cover in dim light but showed no preference in total darkness (Tárano, 1998). Males have little to no control of pair movement once in amplexus because they ride on a female's back until nest construction is completed. Therefore, it is up to females to locomote to oviposition sites. We asked the following question: do females choose oviposition sites based on the presence or absence of conspecific nests?

MATERIALS AND METHODS

The study was conducted in Gamboa, Republic of Panamá, from 19 June to 10 August 1998.

Amplexed pairs were captured between 1930 and 2200 h and were kept in plastic bags in a dark cooler for 1–8 h until tested. Experiments were conducted from as early as 2100 h to as late as 0945 h. Petri dishes (14 cm diameter, 1.5 cm height) were placed at either end of four terraria [measuring 75 × 30 × 30 cm, 50 × 30 × 30 cm, 64 × 31 × 36 cm, and 50 × 26 × 30 cm (L × W × H), respectively]. Terraria were kept in a closet where temperature fluctuation was minimal (27.0 ± 0.8 C; 14 measurements taken over two nights: 9–10 Aug. 1998). Light level was kept constant by blocking the bottom of the closet door with a towel (a researcher standing in the closet could detect no entering light, even during daylight hours). Pairs were kept in constant darkness to limit their ability to see the unnatural surroundings. Individual pairs were placed in the centers of the terraria under small cups for at least 5 min, then uncovered and left undisturbed for a minimum of 3 h. Nest construction takes between 30 min and 1 h (Rand, 1983), and pairs that did not construct a nest within 3 h did not construct a nest after 36 h (pers. obs.) Each female was presented with a choice of two different petri dish treatments. Both dishes were filled with water collected from sites in a drainage ditch in which there were no nests or tadpoles visible at the time of collection. New water was collected approximately once a week in the late evening during collection of amplexed pairs. A day-old conspecific nest was placed in a petri dish at one side of the aquarium. The petri dish containing the nest was switched every day, and the location of the petri dishes in each terrarium was switched every two days to randomize nest presentation and avoid possible location bias. Each nest came from the oviposition tests performed the night before to ensure consistency of nest age. Terraria and petri dishes were carefully cleaned with a sponge and tap water and thoroughly dried between trials.

RESULTS

We found a statistically significant difference in female preference of oviposition site because 21 of 30 females tested chose the petri dish without a nest over the petri dish with a nest ($P = 0.04$; two-tailed binomial test, Zar, 1984). We found no significant effects of petri dish (14 of 25 chose dish 1, $P = 0.69$; two-tailed binomial test, Zar, 1984) or terrarium side (16 of 30 chose the left side of the terrarium, $P = 0.86$; two-tailed binomial test, Zar, 1984).

DISCUSSION

We might expect that females would discriminate between oviposition sites based on nest presence for several reasons. A female who places her nest in puddles without nests may increase her fitness by preventing future competitive interaction (for example, competition for food and space) between her tadpoles and those of the resident nest (Crump, 1991). Additionally, she may avoid inhibition of the growth rate of her offspring because of high tadpole density (Licht, 1967; Alford and Crump, 1982). Reduced growth rate can cause offspring to be smaller in size at metamorphosis (Formanowicz and Brodie, 1982) and may increase the chances that offspring will fail to metamorphose before the temporary pond dries up (Crump, 1991; Wilbur, 1987).

As is true of all studies of animal behavior, motivation of test subjects is always a potential confounding factor. However, females made a clear choice 30 times of 39 trial runs. No female was used more than once in the 11-week study. The statistical strength of the results in light of these factors suggests that the actual tendency to avoid nests during oviposition may be stronger within this population than is revealed by our small sample size. Further studies that more tightly control for seasonal and individual variations in motivation are needed to discover the true strength of the behavior tendency. Although the size of the petri dish did not inhibit nest deposition, it is another possible factor affecting sampling choices made by females. Energy expended in moving among and sampling small ponds where space and resources are limited could be offset by increased reproductive success through avoidance of conspecific competition. However, in large ponds, where space and food are not likely to be limiting, the presence of conspecifics may not lower fitness enough to warrant conspecific nest avoidance. In fact, Ryan (1985) observed communal nest construction by *P. pustulosus* in a large (1 × 2 m) artificial pond and suggested that the lower surface area to volume ratio of communal nests may decrease exposure of eggs to desiccation and predation. It is, therefore, essential to assess the effect of pond size on oviposition site selection behavior. Additionally, observations of frog behavior during the choice of an oviposition site in concert with paired choice tests might reveal the mechanism by which frogs sample potential oviposition sites (see Petranka et al., 1987). Túngara frogs are known to adjust calling behavior in response to ambient light levels (Ryan, 1985), presumably because of the use of

visual cues in predator detection. They may also use visual cues during oviposition site selection since they adjust their behavior depending on ambient light (Tárano, 1998). The genetic basis of the behavior as well as its variation across populations and habitats are other extensions of the study that could help to explain the behavior's existence and maintenance within this túngara frog population.

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