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Effects of inter-pond distance on the breeding ecology of tungara frogs

Abstract Habitat and resource distributions can influence the movement and aggregation of individuals and thus have important effects on breeding behavior and ecology. Though amphibians have been model systems for the study of breeding behavior and sexual selection, most studies have examined breeding behavior within a single pond. As a result, little is known about how inter-pond distance affects breeding amphibians. We studied the effects of inter-pond distance on the breeding ecology of the tungara frog, *Physalaemus pustulosus*, in replicated pond arrays in which distance was varied from 0 to 50 m. We predicted that male site fidelity and male aggregation within arrays would increase with inter-pond distance, and that the opportunity for mate choice and oviposition site selectivity by females would decrease with the distance between ponds. Male site fidelity did increase with inter-pond distance. However, male aggregation decreased with distance, such that males tended to be more evenly spaced among ponds when ponds were farther apart. The opportunity for mate choice by females, measured as the number of males within the phonotactic radius of females, also decreased with inter-pond distance. Each of these three responses was consistent with a threshold effect between 5 m and 10 m in inter-pond distance. This threshold corresponded to the maximum distance at which females in laboratory choice experiments exhibited phonotaxis toward the "whine" call of a tungara male, suggesting that phonotactic limits may play an important role in tungara movements and spacing patterns. The distribution of egg masses among ponds, a potential correlate of oviposition site selectivity, did not vary with inter-pond distance. Multiple egg masses deposited on the same night were significantly overdispersed in all distance treatments, implying that females may select oviposition sites to avoid conspecific egg masses over distances of at least 50 m. Collectively, these results demonstrate that inter-pond distance may indeed affect amphibian breeding and movement behavior, and that consideration of multiple habitat patches and their spatial distributions can provide new insights into even the most well-understood mating systems.

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Introduction

Movement, aggregation, and breeding behavior can vary due to a range of ecological factors (Lott 1984, 1991; Slobodchikoff 1988). Theory suggests that the spatial distribution of resources should be one of the most important influences on behavioral variation (Emlen and Oring 1977), and empirical studies have generally supported the idea that resource distributions can have strong effects on breeding ecology. For example, studies on a variety of taxa have demonstrated that changes in food abundance and distribution (Jarman 1974; Uetz et al. 1982; Rypstra 1989; Travis and Slobodchikoff 1993; Travis et al. 1995) can affect group size, the operational sex ratio, and/or the degree of sexual selection within a population.

In addition to food resources, many species require discrete habitat patches for reproduction. Breeding habitats such as nest sites for birds, burrows for small mammals, and oviposition sites for insects may vary substantially in space and time, and can affect the ways in which reproducing individuals interact. For example, Gowaty and Bridges (1991) showed that higher nest box densities

resulted in increased extra-pair fertilizations in eastern bluebirds (*Sialia sialis*). In other nest box manipulation experiments, Allen and Nice (1952) and Muldal et al. (1985) found that use of grouped nest boxes could result in normally solitary bird species nesting in aggregations. Similarly, Shields et al. (1988) suggested that nest site scarcity promotes coloniality in barn swallows (*Hirundo rustica*).

For anurans, pond distributions may be analogous to avian nest site distributions in their capacity to influence the interactions of breeding individuals. However, most classic studies of anuran behavior have focused on behavior within a single breeding pond. (e.g., Whitney and Krebs 1975; Wells 1977a; Howard 1978; Davies and Halliday 1979; Ryan 1980, 1983). Studies of anurans within multiple ponds have tended to focus on patterns of presence/absence and population turnover (e.g., Sjögren 1991; Sjögren-Gulve 1994; Hecnar and M'Closkey 1996), rather than behavior. As a result, very little is known about how reproducing anurans make use of multiple ponds, and how inter-pond distance affects breeding ecology.

The tungara frog, *Physalaemus pustulosus*, on Barro Colorado Island (BCI), Panama breeds in small ephemeral pools, puddles, and tree holes that vary widely in inter-pond distance. Stream pools used by tungara frogs tend to be spatially clumped, with breeding sites separated by as little as 1 m. Conversely, tree holes and forest puddles used by tungara frogs tend to much more isolated, often separated from other sites by distances of up to several hundred meters (D.M. Marsh, unpublished data). Based on previous studies of tungara frogs and other anurans, we made several predictions about how variation in inter-pond distance (over a scale of 0–50 m) should affect tungara frog breeding behavior.

(1) Male site fidelity should increase as inter-pond distance increases. Many adult frogs exhibit breeding site fidelity (see Sinsch 1990; Blaustein et al. 1994 for reviews), and tungara frogs may be at least somewhat faithful to individual breeding sites (Green 1990). As breeding ponds become more isolated, the cost of inter-pond dispersal should increase. Thus, male tungara frogs should be more likely to remain in the same pond from night to night when ponds are more isolated.

(2) Male aggregation should increase as inter-pond distance increases. When multiple ponds are available, frog distributions within pond arrays may be aggregated (i.e., most frogs are found within a single pond while nearby ponds remain empty), overdispersed (i.e., frogs tend to be evenly distributed among available ponds), or random. Ryan et al. (1981) showed that aggregation can benefit male tungara frogs by increasing the per capita mating success and decreasing the per capita predation rate. However, where breeding ponds are very close together, males will always be aggregated, regardless of their distribution among ponds. Thus, where inter-pond distances are small, there should be little benefit for frogs to aggre-

gate within the same pond. However, where breeding ponds are more isolated, males within separate ponds will be less aggregated with respect to predators or females. As a result, at some inter-pond distance it should become beneficial for breeding males to aggregate within the same pond.

(3) The opportunity for mate choice should decrease as inter-pond distance increases. Mate selection by females based on male call characteristics has been extensively documented for tungara frogs (Ryan 1980, 1983, 1985). Emlen and Oring (1977) argued that when resources used by males are more widely separated, males will themselves be more isolated and females will have less opportunity to select among multiple potential mates. Based on similar reasoning, we predicted that as inter-pond distance increases, female tungara frogs will have less opportunity for mate choice, where opportunity for mate choice is measured as the number of males within the phonotactic radius of females. We note that an increase in male aggregation with increasing inter-pond distance, as predicted above, would tend to offset this effect. However, to the extent that male aggregation may not increase optimally with inter-pond distance, and to the extent that females may not always be able to locate male aggregations, increased distance between ponds will tend to decrease the opportunity for mate choice by females.

(4) Oviposition site selectivity by females should decrease as inter-pond distance increases. Female frogs of several species select oviposition sites to avoid predators of larvae (Resetarits and Wilbur 1989, 1991; Petranka et al. 1994) or conspecific eggs or larvae (Resetarits and Wilbur 1989, 1991; Crump 1993; Spieler and Linsenmair 1997). Growth and development of tungara tadpoles is density dependent in small ponds (D.M. Marsh, unpublished data). Female tungara frogs would therefore be expected to avoid ovipositing in ponds containing conspecifics. The ability of females to select optimal sites, however, depends on their ability to properly sample these sites (Schoener 1971; Kareiva 1982). Because sampling multiple ponds becomes more difficult as inter-pond distance increases, the ability of females to avoid previously used ponds should decrease with increasing inter-pond distance.

We tested each of these four predictions with a field experiment using artificial breeding ponds that were naturally colonized by tungara frogs. We created replicated arrays of three artificial ponds, separated by one of seven distances: 0, 2, 5, 10, 20, 30, or 50 m. We surveyed these experimental ponds over a period of 6 weeks in 1997 and captured and marked all tungara frogs found within them. We used these mark-recapture data to calculate site fidelity, male aggregation, the number of males within the phonotactic radius of each female (i.e., opportunity for mate choice), and the distribution of egg masses among ponds (a correlate of oviposition site selectivity).

We then analyzed the relationship between these behavioral parameters and inter-pond distance to determine the effects of inter-pond distance on tungara frog breeding ecology. We also used a laboratory experiment to determine the spatial scale over which female tungara frogs are attracted to the calls of individual males. These data were necessary to calculate the opportunity for mate choice by females (see above) and to assess the likelihood that phonotactic limits influence tungara frog responses to inter-pond distance.

Materials and methods

Study site and species

Tungara frogs are a small (adult snout-vent lengths of 2.2–3.4 cm), Neotropical species in the family Leptodactylidae. Tungara frogs on BCI, Panama, and the surrounding mainland have been studied extensively, particularly in relation to anti-predator behavior (Ryan et al. 1982), mating behavior (Ryan 1985; Green 1990), and sexual selection (Ryan 1980, 1983; Ryan and Rand 1995). These studies have examined tungara breeding behavior primarily within the laboratory or in two permanent cement ponds on BCI. At these sites, male tungara frogs assemble at breeding ponds shortly after dusk and begin calling. Both females and other males are attracted to these calls. Females select males from within choruses, and the amplexed pair leaves the breeding site. They return to water (though not necessarily the male's calling site) several hours later, and together construct a foam nest containing the fertilized eggs. Amplexed pairs have never been observed to make multiple foam nests in a single breeding bout. In addition, tungara frogs are rarely found at breeding sites when they are not engaging in reproductive activity. They have a prolonged breeding season (sensu Wells 1977b), with most breeding taking place between the months of May and October.

The natural breeding habitats of tungara frogs on BCI are primarily stream pools, puddles, water-filled depressions, and tree holes (Rand 1983 and personal observations). Stream pools tend to be spatially clumped while puddles, depressions, and tree holes tend to be more isolated (D.M. Marsh, unpublished data). However, none of these sites are stable throughout the breeding season, over the course of which, many, particularly stream pools, flood and become largely unsuitable for tungara frog reproduction. At the same time, new sites, particularly puddles and forest depressions, begin to fill and are readily used by tungara frogs. The year of this study, 1997, was an unusually dry year and several of these later-filling sites dried by July or August. Thus, the distribution of suitable breeding sites varies highly in both space and time.

Experimental pond arrays

Each experimental pond array consisted of three ponds arranged at the vertices of an equilateral triangle (Fig. 1). Arrays were assigned one of seven inter-pond distance treatments: 0 m (i.e., pond edges were in contact), 2, 5, 10, 20, 30, and 50 m. We used three replicate arrays for each of these inter-pond distance treatments; thus there were 21 total arrays. We selected sites for arrays such that all arrays were located on relatively flat, forested terrain and at least 100 m from any natural breeding sites or other experimental pond arrays. Inter-pond distance treatments were randomized among these suitable sites.

For experimental ponds we used brown plastic basins with a diameter of 30 cm and a depth of 10 cm, sunk into the ground. These ponds, though small, were within the size range of stream pools, puddles, and tree holes used by breeding tungara frogs. Experimental ponds were stocked with a layer of leaf litter and debris and filled with water from a stream in which tungara frogs were actively breeding. In addition to tungara frogs, experimental ponds
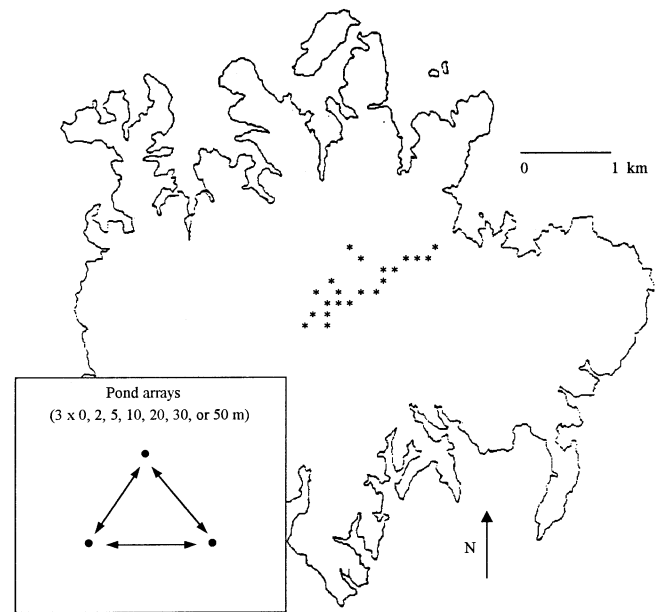

Fig. 1 Map of Barro Colorado Island. Approximate locations of pond arrays are indicated with an *asterisk*. *Offset* A sample pond array with three ponds separated by one of several distances

were colonized by larval *Culex* and *Toxorhynchites* mosquitoes, and tadpoles of *Colostethus nubicola* and *Dendrobates auratus*. Because *D. auratus* tadpoles are predators of tungara frog tadpoles, *D. auratus* tadpoles were removed from ponds when encountered. Other colonizing species were allowed to remain within experimental ponds. However, to ensure homogeneity of ponds within an array, every 4–6 days, all ponds within an array were emptied into a large bucket. This water was mixed and then redistributed among the ponds.

Pond surveys

Ponds were surveyed on 17 nights between 7 July and 13 August 1997. Each survey consisted of one search of all the pond arrays. During a search, all tungara frogs were captured and marked with individual toe-clips (Donnelly et al. 1994). We recorded sex and snout-vent length of captured frogs, and then released frogs at the site of capture. Because most tungara frogs resumed breeding activity (i.e., calling or amplexus) almost immediately upon release, it is unlikely that our activities had any major impacts on tungara frog behavior. Each morning from 8 July to 22 August 1997, we counted the number of foam nests in each experimental pond. We then removed these nests to ensure homogeneity among ponds.

Statistical analysis

In most behavioral studies, individuals are the principle units of analysis, and hypotheses are tested by replicating across individuals. Because of the "snapshot" nature of this study, we were unable to track the decisions made by individual tungara frogs. As a result we test the effects of inter-pond distance on indices of behavior that reflect the mean behavior of all individuals (of each sex) observed within an inter-pond distance treatment. Therefore we are actually testing the effects of inter-pond distance on the average behavior of groups of frogs, not the behavior of individuals. While averaging across individuals does obscure individual variation in response, average behavioral responses are nevertheless highly relevant to behavioral ecology and are commonly incorporated into population and community models (e.g., Turchin 1989; Pulliam and Danielson 1991).

Site fidelity

We defined male site fidelity as the probability that a recaptured male would be found in the same pond in which he was last captured. Based on this statistic, site fidelity should vary between zero for continual movement between ponds and one for complete site fidelity. Random use of the three ponds within an array would yield a site fidelity measure of 1/3. For each inter-pond distance treatment, we calculated site fidelity as the total number of recaptures of males in the pond of previous capture divided by the total number of recaptures of males. We computed one site fidelity estimate for each treatment rather than for each individual array because few data were available for a number of arrays. Furthermore, because some males may have made multiple movements between recaptures, and because some individuals likely dispersed out of the pond arrays, this measure of site fidelity is necessarily relative. We used regression analysis to test the prediction that site fidelity will increase with inter-pond distance. Because site fidelity is a proportion, we arcsine square-root transformed site fidelity estimates to improve normality. Additionally, to account for high variation in sample size, we weighted each estimate for site fidelity by the total number of recaptures at that inter-pond distance. As weighting may render least-squares approximations invalid (Neter et al. 1990), we used maximum likelihood to estimate the regression slope (B_1), and then used a likelihood ratio test to evaluate the null hypothesis that this slope was equal to zero. Subsequent regressions (see below) were also carried out in this manner.

Male aggregation

We used variance-to-mean ratios (Krebs 1989) to measure male aggregation. Variance-to-mean ratios converge to 1 for random distributions of individuals. A variance-to-mean ratio >1 indicates a clumped distribution and a variance-to-mean ratio <1 indicates overdispersion. For each night in which there were multiple males within an array, we estimated male aggregation as the variance in males per pond divided by the mean number of males per pond for that array. Most of these calculations involved small numbers of frogs (i.e., 2–4). For example, if two males were in separate ponds within an array, variance/mean would equal 0.5. If these frogs were in the same pond, variance/mean would equal 2.0. Because sample sizes were small, and because variance-to-mean ratios will tend to have skewed distributions, we averaged all variance-to-mean ratios within an inter-pond distance treatment to obtain a single estimate for aggregation at each inter-pond distance. Again we weighted each of these estimates by total sample size (i.e., number of array-nights for each treatment) in regression analysis. We note here that variance-to-mean ratios tend to increase with sample size (Hurlbert 1990). Thus, use of these ratios depends on the assumption that there is no relationship between inter-pond distance and the amount of male breeding activity within an array. We return to this assumption in the Results section below.

Opportunity for mate choice

Opportunity for mate choice was measured as the mean number of males that each female could assess at any given time. We determined the appropriate radius for mate assessment by females with the laboratory experiment described below. We then calculated the number of males in all ponds within the phonotactic radius of each female captured. We took the mean of these individual measures to obtain an overall index of the number of males/female for each inter-pond distance treatment. We analyzed the effects of inter-pond distance on the opportunity for mate choice with regression analysis and with standard techniques for categorical data.

Oviposition site selectivity

If ovipositing frogs avoid ponds with previously deposited nests, then on nights when there are multiple breeding pairs within an ar-ray, nests should tend to be evenly spread among the available ponds. We thus used the distribution of foam nests within pond arrays as a correlate of oviposition site selectivity. For nights where multiple nests were constructed within an array, we calculated nest distribution as the variance in nests per pond divided by the mean number of nests per pond, where this parameter should be significantly less than 1 if females actively avoid ovipositing in ponds containing conspecific nests. We then analyzed the effects of inter-pond distance on nest distributions within arrays using regression analysis. We were able to use individual arrays as replicates here because sufficient data were available. The use of variance-to-mean ratios to measure nest distributions again requires that there is no effect of inter-pond distance on the total number of nests within an array.

Laboratory phonotaxis experiment

Based on preliminary data, we hypothesized that females would be attracted to the calls of an individual male at a distance of 5 m, but would not be attracted to a male's call at 10 m. We tested this hypothesis with a series of two-speaker choice tests at the laboratory of the Smithsonian Tropical Research Institute in Gamboa, Panama (see Ryan and Rand 1995). Female tungara frogs were captured in amplexus early in the evening, and tested and released the same night. Each female was placed under a funnel in the center of the 2.7×1.8 m acoustic chamber and midway between two speakers. The chamber was illuminated with infrared radiation. One speaker played, every 2 s, the whine call of a male tungara frog at either 70 dB sound pressure level, approximately equivalent to a male call 5 m away, or at 64 dB, approximately equivalent to a male call 10 m away (based on a rate of sound attenuation of 6 dB per doubling of distance; Beranek 1954; Ryan 1985). The opposite speaker played a burst of white noise with a whine envelope alternately with the call. For successive females, calls and noise were played from opposite speakers. After 3 min, the funnel was raised from outside the chamber and the female was allowed to move freely. Her movements were observed with an infrared-sensitive video camera. A female was scored as exhibiting phonotaxis if she moved to within 10 cm of the speaker that played tungara calls. A female was scored as not exhibiting phonotaxis if she did any of the following: remained at the release point for 5 min, subsequently remained motionless for 2 min, approached to within 10 cm of the "white noise" speaker, or did not approach either speaker within 15 min. In addition, the motivation of each female was tested with a standard tungara whine call at 90 dB, equivalent to a distance of 0.5 m. Females were considered as motivated if they exhibited phonotaxis to the standard call in a screening trial both before and after the experimental trial. Twenty motivated females were scored for each distance treatment, and unmotivated females were not incorporated into the analysis. We used a one-tailed binomial test to compare the observed frequency of phonotaxis to an empirically derived null frequency of 0.10. This null frequency represents the probability that a female tungara frog will exhibit phonotaxis towards a speaker playing white noise when both speakers play white noise.

Results

Site fidelity

Male site fidelity ranged from 0.41 for ponds separated by 5 m to 0.91 for ponds separated by 50 m (Fig. 2). A weighted linear regression indicated that site fidelity did increase with inter-pond distance (B_1=0.10, df=1, $P_{(B1=0)}$=0.008). However, inspection of the graph shown in Fig. 2 suggests that tungara frog site fidelity exhibited a threshold response to inter-pond distance. Within ar-
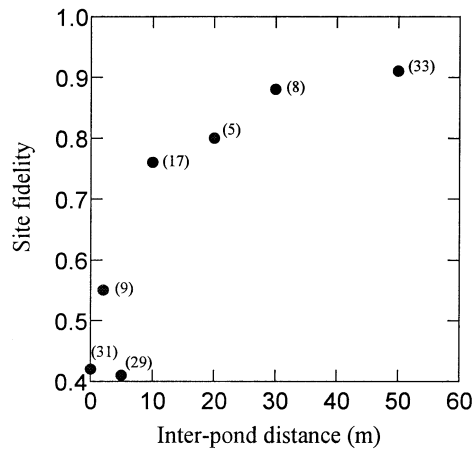
Fig. 2 Effects of inter-pond distance on the site fidelity of male tungara frogs. *Values in parentheses* indicated sample sizes in terms of the total number of recaptures at each inter-pond distance treatment
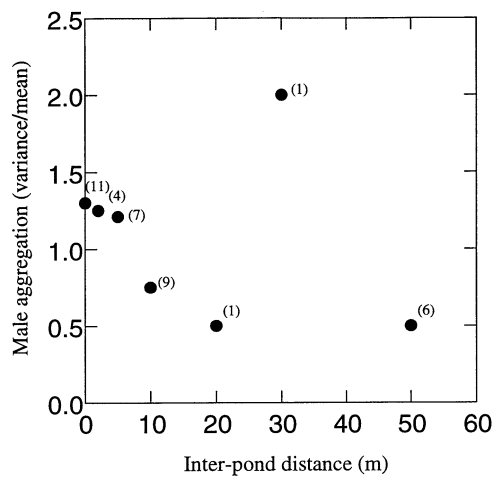

Fig. 3 Effects of inter-pond distance on the aggregation of male tungara frogs. Aggregation was measured as the variance in frogs per pond within an array on a given night divided by the mean number of frogs per pond within the array on that night. *Values in parentheses* indicate sample sizes in terms of the number of array-nights used to calculate aggregation for each inter-pond distance treatment

Table 1 Pairwise *G*-tests for heterogeneity in male site fidelity at variable inter-pond distances. Results indicate that site fidelity was similar within ponds separated by 0–5 m, and within ponds separated by 10–50 m, but was significantly lower within ponds separated by 5 m than within ponds separated by 10 m

Comparison	Site fidelity data (recapturess in same pond/recapturess)	df	G	P
0 m vs 2 m	13/31 vs 5/9	1	0.51	0.47
2 m vs 5 m	5/9 vs 12/21	1	0.56	0.46
5 m vs 10 m	12/21 vs 13/17	1	5.54	0.019*
10 m vs 20 m	13/17 vs 4/5	1	0.03	0.87
20 m vs 30 m	4/5 vs 7/8	1	0.13	0.72
30 m vs 50 m	7/8 vs 30/33	1	0.08	0.78

rays of ponds separated by 5 m, tungara frogs were recaptured in the same pond 41% of the time, only slightly more likely than would be predicted if pond use were random. In contrast, within arrays of ponds separated by 10 m, 78% of all recaptures were at the site of previous capture. That this can be viewed as a threshold response is supported by the fact that this difference in frequency of site fidelity (between 5-m arrays and 10-m arrays) was significant (*P*<0.02, see Table 1), while differences in site fidelity were non-significant for all other pairs of similar inter-pond distance treatments (Table 1). Although the significance level for the former comparison (5–10 m) is somewhat liberal considering that we are making multiple comparisons, we do have a biological justification for expecting a threshold between these treatments (see results for Laboratory phonotaxis experiment, below). In addition, the frequency of site fidelity in arrays of 5 m or less was significantly different from that in arrays of 10 m or greater (*G*=26.90, *df*=1, *P*<0.0001). Unfortunately, rigorous testing of more explicit threshold models requires more degrees of freedom than were available for this analysis.

Male aggregation

Contrary to the a priori prediction, male aggregation tended to decrease as inter-pond distance increased (Fig. 3). A weighted linear regression of aggregation on inter-pond distance was marginally significant (B_1=–0.014, *df*=1, $P_{(B1=0)}$=0.10), though the estimated aggregation at 30 m was a strong outlier and based on only one observation of two males within the same pond. The graph in Fig. 3 is also consistent with a threshold for aggregation between 5 and 10 m. Variance-to-mean ratios for male aggregation in arrays of 0–5 m were significantly higher than variance-to-mean ratios in arrays of 10–50 m (Mann-Whitney *U*-test, *U*=102.0, *P*=0.019). There was no detectable effect of inter-pond distance on the total number of observations of males within an array (B_1=–0.010, *df*=1, $P_{(B1=0)}$=0.92) which substantiates the use of variance-to-mean ratios for the analysis of male aggregation.

Opportunity for mate choice

For the calculation of the number of males that each female could assess at any given time, we used a radius of 5 m (see below for justification). The number of potential mates per female assuming this phonotactic radius is shown in Fig. 4. A linear regression of males/female on inter-pond distance provides a poor fit to the data (B_1=–0.012, *df*=1, $P_{(B1=0)}$=0.15). Rather, the data appear to be more consistent with a threshold for the effects of inter-pond distance between 5 and 10 m. We thus categorized the number of potential mates per females as 0, 1, or >1, and compared the distribution of these categories among females in arrays of 5 m or less and females in ar-
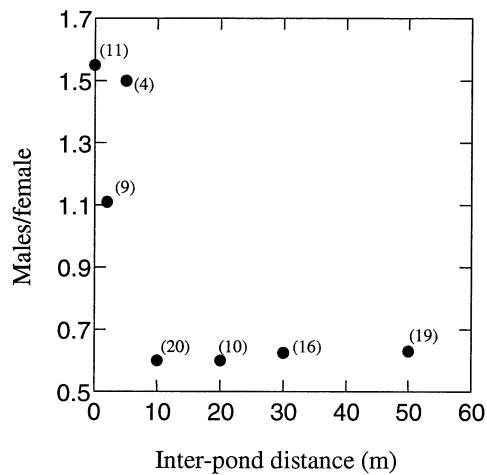
Fig. 4 Effects of inter-pond distance on the opportunity for mate choice, measured as the number of males in ponds within 5 m of each female. *Values in parentheses* indicate sample sizes in terms of the numbers of females at each inter-pond distance treatment

Table 2 Comparison of opportunity for mate choice in ponds separated by 0–5 m versus ponds separated by 10–50 m. The table indicates the numbers of females with at least two potential mates, one potential mate, and no potential mates for each inter-pond distance category

Inter-pond distance	Potential mates			G	P
	≥2	1	0		
0–5 m	11	10	3	22.34	0.0001
10–50 m	3	34	28		

rays of 10 m or more (Table 2). The distribution of these categories among inter-pond distance classes was highly heterogeneous (G=22.34, df=2, P=0.0001), with females having significantly fewer potential mates in the more highly dispersed ponds.

Oviposition site selectivity

There was no detectable relationship between nest distribution and inter-pond distance (B_1=0.000, df=1, $P_{(B1=0)}$=0.92; Fig. 5). Given the low standard error (0.002) for the regression coefficient, this test was reasonably powerful. A post hoc power analysis (Neter et al. 1990) indicated that a B_1 of 0.01 (corresponding to a gradual decline in selectivity with inter-pond distance to no selectivity at 50 m) would have been detected with a probability of 0.97. The lack of effect of inter-pond distance on nest distribution corresponded to significant overdispersion of nests (i.e., variance/mean significantly<1) in all inter-pond distance treatments (one-sample t-tests, P<0.05 in all cases; Table 3), consistent with the avoidance of conspecific nests by ovipositing females. For arrays in which ponds were separated by distances of 10 m or greater, the fact that males were also overdis-
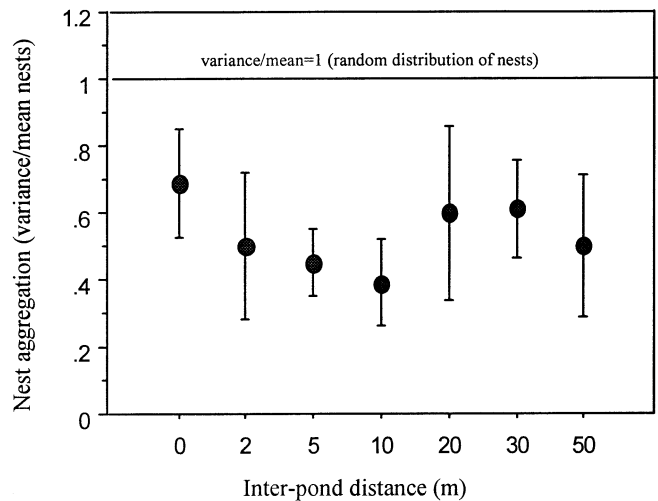

Fig. 5 Effects of inter-pond distance on the distribution of foam nests among ponds. Nest distribution was measured as the variance in nests per pond within an array on a given night divided by the mean number of nests on that night. The mean±2 SE are shown. There was no effect of inter-pond distance on nest distribution; nests were significantly overdispersed (variance/mean<1) in all treatments

Table 3 Mean nest aggregation (variance-to-mean ratio, v/m) for each inter-pond distance treatment. One sample t-tests were used to test the hypothesis that dispersion is significantly different from 1.0 (the variance-to-mean that would be expected with random dispersion of nests)

Inter-pond distance (m)	Mean v/m	df	t-value	P (mean=1.0)
0	0.686	10	−3.85	0.0032
2	0.500	6	−4.58	0.0038
5	0.450	9	−11.00	<0.0001
10	0.389	17	−9.46	<0.0001
20	0.599	8	−3.10	0.0146
30	0.611	8	−5.29	0.0007
50	0.500	11	−4.69	0.0007

persed to some extent complicates the interpretation of this result. However, the average of the variance-to-mean ratios for nests in these larger arrays was 0.498, which was still significantly lower than the variance-to-mean ratio expected on the basis of male overdispersion alone (0.753), (one-sample t-test, t=−5.54, df=47, P<0.001). This latter result suggests that at least some oviposition site selection to avoid conspecific egg masses occurs over the range of spatial scales considered in this experiment. There was no effect of inter-pond distance on the total number of nests within a pond array (B_1=0.046, df=1, $P_{(B1=0)}$=0.70), again substantiating the use of variance-to-mean ratios in this analysis.

Laboratory phonotaxis experiment

A significant proportion of female tungara frogs approached a male call that had an intensity equivalent to

calls at 5 m (8 moved toward the speaker, 11 did not, P=0.0002). The proportion of females that approached the speaker playing the 10 m call was not statistically different from the null frequency of 0.10 (2 moved toward the speaker, 18 did not, P=0.58). Thus females appear to exhibit phonotaxis to a tungara whine call at 5 m but not at 10 m.

Discussion

Inter-pond distance had marked effects on the observed breeding behavior of tungara frogs. Both male site fidelity and male aggregation showed responses consistent with a threshold between 5 and 10 m. In arrays of ponds separated by 5 m or less, males moved frequently among ponds and multiple males tended to aggregate within the same pond. In arrays of ponds separated by 10 m or greater, males remained somewhat site faithful from night to night and tended to spread themselves among the available ponds. Because the data for site fidelity and aggregation are derived from the same individuals' movement patterns, these data should be viewed as two results of the same underlying set of behaviors. Determining the precise mechanisms that produce the observed responses would require more complete knowledge of the perceptual abilities of tungara frogs, as well as a better understanding of the costs and benefits of moving and aggregating over a range of spatial scales.

We can, however, offer two general hypotheses that may partially explain the results for site fidelity and aggregation. First, tungara males, like females, may be attracted to the call of another male at 5 m but not at 10 m. Thus, males might often leave one pond to join a male in a nearby (i.e., 5 m) pond, but not leave a pond when the nearest male is farther away. This would explain both the increased site fidelity and the decreased aggregation at inter-pond distances of 10 m and above. Additionally, male spacing and movement behavior might be a strategic response to the phonotactic limits of females. As females do not exhibit phonotaxis at 10 m, an individual male that chose his own pond in the 10 m or larger arrays might avoid direct mate competition with males in neighboring ponds. This is likely to be adaptive in some circumstances, and would again explain both the increased site fidelity and the decreased aggregation in the larger arrays.

Both of these hypotheses assume that active male behavior is responsible for generating the observed effects. It is also possible that the increase in site fidelity and the decrease in aggregation with inter-pond distances result from the inability of male frogs to find ponds at distances greater than 5 m. However, several lines of evidence appear to be inconsistent with this conclusion. First, many male tungara frogs disperse at least several hundred meters over the course of the breeding season, and sometimes make multiple movements between ponds over these distances (Marsh et al. 1999). Second, all ponds within experimental pond arrays were rapidly colonized by tungara frogs. Given these high levels of dispersal and colonization, it would be surprising if locating a pond at 10 m presented a great difficulty for tungara frogs. The use of olfaction to locate breeding sites has been widely reported in amphibians (e.g., Savage 1961; Oldham 1967; Joly and Miaud 1993), as has directed dispersal towards breeding sites from distances up to several kilometers (Twitty et al. 1964; Oldham 1967; Dole 1968; Sjögren-Gulve 1998).

Marked changes in the number of males within the phonotactic radius of females were also observed between the 5- and 10-m treatments for inter-pond distance. This result was partly due to the fact that males tended to be overdispersed in arrays of 10 m or greater, making it unlikely that females would encounter multiple males at any one pond. Changes in the opportunity for mate choice may also have been due to the limited ability of females to locate an isolated male at distances beyond 5 m. In any case, these data suggest that the opportunity for mate selection by females may depend on the distribution of suitable breeding habitat. The strength of sexual selection may therefore vary within and between populations as a result of variation in breeding pond distribution. It should be cautioned, however, that female tungara frogs may assess multiple males by moving between ponds. Indeed, the relatively high mobility of females is suggested by the overdispersion of egg masses over larger spatial scales. If females do indeed move among more distant ponds to find mates, then females with few males nearby would find it more difficult to assess multiple potential mates; they would not, however, be entirely prevented from doing so. The difficulty in knowing which males may be considered as potential mates highlights the need to consider spatial scale explicitly in measures of the operational sex ratio (OSR). In most studies, OSRs are measured somewhat arbitrarily on the scale of the study site, rather than on a scale that is known to be relevant to breeding individuals (Gwynne et al. 1998).

Although male movement patterns and female mating opportunities varied with inter-pond distance, the tendency of females to distribute their nests among available breeding sites was not affected by inter-pond distance over the spatial scales examined. Foam nests were in fact significantly overdispersed in all inter-pond distance treatments. This overdispersion of foam nests suggests that some oviposition site selection probably occurs over distances of at least 50 m. Active oviposition site selection over this distance is particularly impressive since we considered oviposition patterns only within a single night. As some amplexing pairs may not oviposit until subsequent nights (D.M. Marsh, unpublished data), and some females may forgo breeding if suitable ponds are not available, avoidance of conspecific nests could potentially occur over a much larger scale.

Results from the laboratory phonotaxis experiment confirmed our original hypothesis that the range over which females are attracted to the whine calls of individual males is indeed quite small (<10 m). Although we

cannot be certain that the observed range of phonotaxis is responsible for the threshold changes in male spacing and movement behavior, the concordance of the spatial scales of the two phenomena is certainly suggestive of a relationship. One should not assume, however, that 5 m necessarily represents the maximum distance at which females can hear a male's call. It is difficult to separate signal reception from motivation in a laboratory experiment such as this. In addition, preliminary data (A.S. Rand, unpublished data) suggest that the calls of males are in fact audible to females at a somewhat greater distance, and that females may respond to a more complex "whine and chuck" call at a distance of 10 m. It is also likely that the calls of choruses may be heard at greater distances than the calls of single males (Wells 1977b). Thus, our results for female phonotaxis may not apply in situations where per pond frog densities are high and multiple frogs are present in most ponds. These phonotaxis results are still, however, highly relevant to the inter-pond distance experiment described above, as 92% of ponds containing males in this experiment contained only a single male.

We have presented our results primarily in terms of a test of the effects of inter-pond distance on tungara frog behavior. However, these results could also be viewed in the context of determining the spatial scale over which different behaviors are likely to be important for the ecology of a species. The importance of explicit consideration of spatial and temporal scales has received extensive discussion in the ecological literature (see Kareiva and Wennegren 1995; Levin et al. 1997 for recent reviews); it has received substantially less attention in the literature of animal behavior and behavioral ecology. Though it may be a truism that all behavior is ultimately scale dependent, knowing the scale over which different behaviors are observed is important for understanding the role of those behaviors in the ecology of a population. For example, male tungara frogs appear to aggregate only over very small scales when multiple ponds are available. This suggests that aggregation behavior should not affect patterns of abundance on a large scale (e.g., several hundred meters). Aggregations that are observed on this larger scale are thus likely to result not from attraction to conspecifics, but from individual responses to habitat quality or philopatry. The scale at which females are attracted to the calls of males also has important implications for the ecology of tungara frogs. That females exhibited phonotaxis only over small scales suggests that they may not use the calls of males to locate breeding sites. Though this result may depend on the density of calling males, the observation of lone females at experimental breeding ponds provides additional evidence that many females locate and assess breeding ponds independently of the locations of calling males. Ultimately, though, a detailed understanding of the role of this sort of behavioral variation in ecology and population dynamics will require explicit experimental manipulations of behaviors of interest. Nevertheless, identification of the scales over which behaviors affect patterns of habitat use can provide a good starting point for these experiments.

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