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UNIVERSITY OF OKLAHOMA
GRADUATE COLLEGE

PRIVATION AND UNCERTAINTY IN THE SMALL NURSERY OF PERUVIAN LAUGHING FROGS: LARVAL ECOLOGY SHAPES THE PARENTAL MATING SYSTEM

A Dissertation
SUBMITTED TO THE GRADUATE FACULTY
in partial fulfillment of the requirements for the
degree of
Doctor of Philosophy

BY

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Norman, Oklahoma
2002

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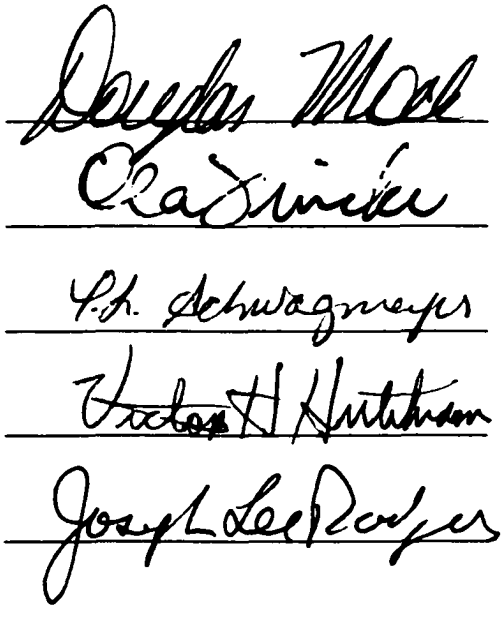
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PRIVATION AND UNCERTAINTY IN THE SMALL NURSERY
OF PERUVIAN LAUGHING FROGS: LARVAL ECOLOGY
SHAPES THE PARENTAL MATING SYSTEM

A DISSERTATION APPROVED FOR THE
DEPARTMENT OF ZOOLOGY

BY



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With the help of many friends, colleagues, and funding organizations I was able to fulfill one of my life goals – to live for an extended time in a tropical rainforest. For that opportunity I will be forever thankful.

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And now, I believe..I...am....finally.....finished.

angel

thanks for introducing the laughing frog to me

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FEMALE REPRODUCTIVE STRATEGY IS SHAPED BY LARVAL ECOLOGY IN A BROMELIAD-BREEDING TREE FROG

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ABSTRACT

I investigated the life history traits of a Neotropical hylid, *Osteocephalus planiceps,* in relation to the stochastic nature of its larval microhabitat. This anuran deposits eggs in the water-filled leaf axils of bromeliads and palms. To determine the features that allow this species to exploit such larval habitats, I studied a free-living population of *O. planiceps* within a 3600 m^2 area of the upper Amazon Basin, Departamento Loreto, Peru that contained both natural and artificial oviposition sites (water-filled plastic cups). The artificial sites allowed me to track survivorship of larvae and reproductive effort of breeding females (as frequency of egg depositions and clutch sizes). I also conducted laboratory experiments to determine how egg density affects tadpole survivorship, developmental time, and body size at emergence. Each female distributed 3 to 5 subsets of her clutch across several catchments, depositing eggs according to water volume (ca 2 - 2.5 eggs/ml). Clutch size increased with inter-clutch intervals and females adjusted their inter-clutch intervals to rainfall amounts by preferentially ovipositing on days after substantial rainfalls, when water oxygen levels are highest in the catchments. When choices were available, females tended to oviposit in larger volume nurseries and their egg masses fared better in such sites. Throughout tadpole development, females returned periodically to the oviposition sites, paired with males, and deposited additional fertilized eggs. Most of these secondary eggs were eaten immediately by resident tadpoles. In addition to oophagy, tadpoles were cannibalistic on siblings. Cannibalism appeared to be density-dependent. Tadpole density declined with larval cannibalism, resulting in more available food (i.e., nutritive eggs) per tadpole. Larval mortality was extremely high due to factors other than cannibalism (e.g.,

heterospecific predation). When a clutch failed, or when all individuals had metamorphosed, the subsequent deposition of eggs by the female essentially became the new primary clutch. The reproductive strategy of this species differs from most other anuran trophic feeders in that the secondary eggs are fertilized. Through over-production of young, females provide a food source for subsets of their initial clutches and automatically create replacement offspring in the event of unpredictable loss.

Key words: anuran, bromeliad, cannibalism, development, larvae, oophagy, Osteocephalus, *over-production, parental care, reproduction*

INTRODUCTION

For the many life forms that require aquatic conditions during embryogenesis and larval development, small and often ephemeral catchments of rainwater offer an important alternative to the permanent standing waters of ponds, lakes, and rivers. A great diversity of aquatic nurseries (i.e., restricted bodies of water in which larvae develop: Mock and Parker 1997) exist, ranging from earthen puddles to plant catchments ('phytotelmes') such as water-filled tree holes and leaf axils. Their patchy distribution and limited size simultaneously reduce the danger from many kinds of predators, but factors such as exposure to light and wind, rainfall, debris accumulation, and various biotic challenges can produce aquatic environments that exhibit tremendous chemical and physical variations (Laessle 1961). In addition, the food base for developing larvae within such closed systems is often limiting. For taxa in which females oviposit repeatedly in the same catchment, both the food

and chemistry problems are likely to be exacerbated by sporadic additions of rivals (see Fincke 1992, 1998). The stochastic nature of these habitats creates complex selection pressures on the life history and behavioral traits of taxa using them as nurseries (Wilbur 1980).

For species whose embryos and/or larvae respire aerobically, females should oviposit in sites with the highest concentration of dissolved oxygen. Accumulation of leaf litter inhibits gas exchange and decomposing organic material may further deplete dissolved oxygen concentrations in the leaf axils (Laessle 1961). Females of some species have a keen ability to assess the chemical and physical properties of the oviposition sites and/or actively remove leaf litter from the sites (e.g., Jamaican bromeliad crab, *Metopaulias depressus*; Diesel 1989), while others may simply choose sites with little or no leaf litter. However, dissolved oxygen depletes rapidly in small volumes, especially when crowded with many respiring larvae, and is replenished primarily by rainfall. Accordingly, there are likely to be sporadic and unpredictable windows of opportunity arising amid conditions that produce total brood loss (Fincke 1999); females are expected to oviposit at times most favorable in terms of dissolved oxygen concentrations such as after rainfall when oxygen levels are highest.

Rich food sources are available for many organisms that live in water-filled depressions in the ground, in tree holes, or in leaf axils (Laessle 1961, Maguire 1971, Frank 1983, Kitching 1987). For some taxa that use these habitats for oviposition, the larvae are able to subsist on detritus, algae and/or micro-organisms (e.g., naidid oligochaetes, psychodid and helodid larvae, Frank 1983; mosquito larvae, Fish and Carpenter 1982; anuran larvae, reviewed in Lannoo et al. 1987). Though for some, the available resources are not sufficient to support developing

larvae. These taxa have evolved diverse ways to supply their young with the necessary nutrients allowing them to exploit impoverished habitats.

The bromeliad crab provides extrinsic food (i.e., snails, millipedes) to developing larvae (Diesel 1989), while some anuran species deposit a few large ova with enough yolk to provide larvae with intrinsic nutrition through metamorphosis (e.g., phytotelme breeding microhylids *Anodonthyla boulengeri*, *Platyhyla grandis*, and *Plethodontohyla notosticta*, Blommers-Schlösser 1975; *Syncope antenori*, Krügel and Richter 1995). A uniquely anuran method that is relatively common in species with arboreal larvae is one in which females provide unfertilized trophic eggs throughout development to a modest number of tadpoles (e.g., 1 - 2 larvae per leaf axil in *Dendrobates pumilio*, Weygoldt 1980, Brust 1993; *D. vanzolinii*, Caldwell 1997). In some species that provide trophic eggs, initial clutches deposited contain a high percentage of unfertilized eggs that tadpoles may consume at hatching (e.g., *O. brunneus*, Lannoo et al. 1987; *Anotheca spinosa*, Jungfer 1996; *Chirixalus eiffingeri*, Kam et al. 1996). Another strategy found in species using food-poor nurseries is to place greater numbers of young in the nursery and allow sibling cannibalism to achieve the optimal balance between supply and demand (van den Bosch et al. 1988; e.g., damselflies, Fincke 1994). Thus, sibling cannibalism has been interpreted as part of a parental strategy for allocating resources to favored offspring (an alternative to producing fewer but larger eggs), the so-called 'icebox hypothesis' (reviewed in Mock and Parker 1997).

One suggested advantage of cannibalism is that carnivory can accelerate development (Crump 1990, Baur 1992), thereby truncating exposure to other stochastic risks. On the other hand, the ingestion of a sibling eliminates one potential

vehicle for inclusive fitness (Hamilton 1964) and may carry additional risks associated with concentrating pathogens (Pfennig 1997, Pfennig et al. 1998). Sib-cannibalism, therefore, is predicted to occur when: a) sibs are available and ingestion of sibs is a practical option (e.g., size asymmetry, Polis 1981, Elgar and Crespi 1992; Fincke 1994); b) alternative food levels are low and/or the potential acceleration in growth that can be obtained is high (Fox 1975, Polis 1981); and c) victim's reproductive value is relatively low (Eickwort 1973, Milinski 1978, Charlesworth and Charnov 1980).

Another stochastic element that frequently wipes out whole broods in these water catchments is predation. While the isolated and sometimes ephemeral sites offer protection from many predators, their limited size offers little escape for eggs and larvae once discovered. One defense against predation is for the female to assess the nursery for pre-existing predators and either avoid the site if predators are present or kill them before ovipositing. For example, *M. depressus* adults search for and kill damselfly larvae before releasing their own offspring (Diesel 1989). Another defense exhibited by a diversity of taxa is to guard eggs and/or developing larvae (e.g., *Eleutherodactylus coqui*, Townsend et al. 1984; *M. depressus*, Diesel 1989).

I present information on larval growth and development in the laughing frog, *Osteocephalus planiceps* (Hylidae), a Peruvian phytotelme-breeding tree frog, with respect to an unusual pattern of parental care described also in a congener, *O. oophagus* (Jungfer and Schiesari 1995, Jungfer and Weygoldt 1999). Specifically, I relate a peculiar combination of life-history traits (relatively large clutch size, use of fertilized nutritive eggs, and virtually continuous breeding) to some of the abiotic (rainfall) and biotic (predation) challenges this frog faces. These forces appear to

have played important roles in shaping the mating system and parental care habits (Haugen, Chapter 2), in addition to having consequences considered here for larval development.

My objectives for this study were to explore: 1) whether females adjust clutch size to nursery volume; 2) the factors that contribute to clutch survival and failure; 3) the effects of rainfall and embryonic respiration on the dissolved oxygen concentration in the nursery and whether females adjust inter-clutch intervals to oviposit when conditions are most favorable in terms of available oxygen; 4) the factors that predict the onset and extent of sib-cannibalism; 5) the possible impact that provisioning developing tadpoles with fertilized 'trophic' eggs has on offspring development and survival; and 6) the consequences of this reproductive strategy on the reproductive success of adult males and females.

METHODS

Study site

I conducted this study in the laboratory clearing of the Amazon Conservatory of Tropical Studies (formerly the Amazon Center for Environmental Education and Research) located in the aseasonal rain forest of the upper Amazon Basin, Departamento Loreto, Peru (03°58'S, 72°59'W), Feb. 1995 to May 1995 and Sept. 1995 to May 1996. The study area consisted of a 3600 m^2 area of controlled secondary growth habitat surrounded by primary forest.

Five years prior to this study, bromeliads were relocated from nearby fallen trees and tied to trees in the laboratory clearing. By 1995, these bromeliads had

established themselves in the area by producing offspring that had attached naturally to the trees. To increase the number of oviposition sites and to facilitate sampling, I attached artificial catchments (plastic cups, see below) to trees throughout the study area. Roughly 45 bromeliads from various taxa (*Aechmea chantinii, A. hoppii, A. nallyi, A. nidularioides, Billbergia* sp., *Guzmania calothyrsus, Guzmania* sp., *Neoregelia eleutheropetala*, and *Werauhia gigantea*) and 12 artificial (i.e., cup) sites were available in the study area. Oviposition sites (bromeliad and cup sites) were patchily distributed and all were located 0 - 3 m above ground.

Preliminary observations indicated that for each oviposition event (ca once a week) females divide their clutches into separate sub-clutches by depositing eggs in 3 - 5 leaf axils of single bromeliads. The leaf axils that females used for oviposition contained little, if any, leaf litter and held different volumes of water (25 ml - 200 ml), both within and between bromeliads. Each artificial site, therefore, consisted of five 250-ml clear plastic cups containing various volumes of water. Each plastic cup was placed within a separate (700-ml) green plastic cup. The larger cups were wired permanently to trees at various angles so that the pools of water in the smaller cups approximated those within the bromeliads. The larger cups thus functioned as holders for the transparent smaller cups and facilitated the counting of eggs deposited and tadpole survival by allowing me to remove the smaller cups easily while maintaining the exact location and position of the inserted cups. Each artificial site was comprised of five cups containing the following volumes of water: 25-ml (one cup), 50-ml (two cups), and 100-ml (two cups). Water volumes in all cups were kept constant by overflow holes in the smaller cups and water added (daily, as needed) when levels fell below these holes.

Field data collection

Each night throughout the study period (273 nights total) from 1800 h - 0200 h I censused the study area and recorded the locations of all males. When an individual frog (male or female) was captured the first time, I recorded mass and snout-vent length (SVL). For individual identification, I made drawings of each frog's unique dorsal markings. In addition, I clipped off the toe pad of the inner toe (digit I) of one hind limb (left or right) to insure the individuality of the dorsal markings and to identify previously captured individuals.

All oviposition sites (artificial and natural bromeliads) were checked at least twice per hour until 0200 h. When an arriving female was detected, I increased my visit frequency to her area to determine both the exact oviposition site and identity of the amplexing male. Females always arrived before 0200 h and rarely mated later than 0200 h. If a female had not deposited eggs by 0200 h, I checked back every half hour, throughout the entire night if necessary, until she did. I counted all eggs deposited in the artificial oviposition sites. I considered a female's clutch to be all eggs deposited by her in a single night and because clutches were typically distributed among several catchments, I will refer to eggs deposited in separate catchments as 'sub-clutches' ('sub-broods' when referring to tadpoles). When a newly oviposited sub-clutch was the 'initial sub-clutch' (i.e., no previous eggs existed in the catchment), I waited until the following day to count the eggs (so as not to disturb the developing eggs). But for sub-clutches deposited into cups already containing tadpoles (hereafter 'secondary sub-clutches'), I counted eggs immediately after the female's departure because resident tadpoles begin consuming such eggs as they were deposited. In addition, I counted the number of eggs deposited in leaf axils ($n = 14$) of natural bromeliads to determine whether numbers of

eggs deposited in the artificial sites were comparable to those deposited in bromeliads. To determine the amount of water in the leaf axils of the bromeliad, I first removed eggs and then extracted the water using a rubber tube attached to a turkey baster. Because water volume in leaf axils varied greatly, depending on rainfall, evaporation, and loss of water due to animals exploiting the resource (including uptake of water by the eggs), I approximated the measured volume in each leaf axil to one of eight volume categories: 25, 50, 75, 100, 125, 150, 175, and 200 ml. Each category included measured volumes of ±12.5 ml.

Throughout this study, 15 different females deposited their clutches in the artificial oviposition sites repeatedly, for a total of 125 oviposition events (mean number of clutches per female = 6.3 ± 3.8, range 2 - 15). Females left the study area immediately after mating, and each female returned to her original site to deposit subsequent clutches. Although ten additional females deposited eggs in artificial sites during the study (and data from these females' sub-clutches are included in the analyses of tadpole survival from the various cup volumes), only those known females that deposited clutches exclusively within the set of five cups at their oviposition sites are included in the clutch size analysis. Thus, I excluded any females that used bromeliads in addition to artificial sites for oviposition because I was unable to ascertain their total clutch size accurately. Of the focal clutches, I was able to count the total number of eggs deposited (before eggs were consumed by tadpoles within the cups) in 74.4% (93/125) of the oviposition events. I did not differentiate between initial clutches and secondary clutches for this calculation because each clutch was divided into sub-clutches and the classification of the sub-clutches (initial vs. secondary) depended upon the presence or absence of tadpoles

in each catchment.

I calculated mean number of eggs deposited in each of the three different volumes of water available to females at the artificial oviposition sites. I did not average zeroes into a female's mean for a particular volume of water if, on occasion, she did not deposit eggs in a cup of that volume because I wanted a true indication of the number of eggs she deposited per volume. However, if a female never deposited eggs in a particular volume, a zero value for that female was averaged in with the means of the other females for the overall mean of that volume. To determine if females deposited numbers of eggs differentially depending upon the presence or absence of tadpoles within the catchments, I compared the number of eggs in initial and secondary sub-clutches per female in the 50-ml cups. The 50-ml cups were chosen because I had more initial sub-clutches recorded in these cups (due to the higher mortality) than I had for the 100-ml cups. Two females were excluded from this analysis because for both I had data on number of eggs deposited in initial sub-clutches only.

Tadpoles began to hatch ca 36 h after egg deposition and by 3 - 4 d post-oviposition all surviving tadpoles in a brood had hatched and were free-swimming. I did not disturb the developing embryos until 4 d post-oviposition; the number of tadpoles present at that time was used to represent hatching success for the clutch. From that point on, I kept daily records of tadpole survival through metamorphosis in the artificial sites. To compare hatchling success and percent survival among the different cup volumes, I trimmed the sample of ovipositing females to those that had deposited eggs in all 3 volumes. Because only one sub-clutch in the 25-ml cups produced froglets, I omitted that volume from the analysis comparing the ratio of sub-clutch size to number of tadpoles surviving through metamorphosis.

Tadpoles consumed dead conspecifics and were seen to actively cannibalize one another. Causes of mortality due to reasons other than cannibalism were recorded when detected. I considered tadpoles to have died from starvation when I had data revealing lack of food (i.e., no trophic eggs available) and observations that the tadpoles were exhibiting no growth and appeared emaciated (visible reduction in postchondrocranial mass) prior to death. Death from asphyxiation occurred following depositions of unusually large number of eggs (most often by more than one female) causing tadpoles (and embryos) to die en masse. Losses due to predation were inferred by the presence of known predators (e.g., snakes, dendrobatid tadpoles).

As tadpoles metamorphosed, I measured the sizes (SVL) of all newly emerged froglets. In addition, froglets emerging from natural bromeliads were measured on an opportunistic basis to determine whether those developing in artificial sites were similar to those from natural bromeliads.

To determine the effects of rainfall and egg deposition on the physical environments of the nurseries, I recorded dissolved oxygen concentration in both natural bromeliads and artificial sites. Using the Checkmate Modular Testing System, I took readings daily at 0800 h in one representative leaf axil for each of 7 bromeliads for 21 d and in 24 100-ml cups at 12 artificial oviposition sites for 51 d (each artificial site had two cups that served as replicates). Of the 12 available artificial oviposition sites, 10 were in use during the measurements of dissolved oxygen concentrations (data from the two unused sites were excluded from the analyses). I recorded rainfall daily throughout the study.

Egg depositions were recorded for both artificial and natural (i.e., bromeliads) oviposition sites throughout the study periods. To determine both the effects of

rainfall on timing of oviposition and oxygen concentrations in the oviposition sites and the subsequent oxygen depletion due to respiring embryos, I selected days with substantial amounts of rainfall in relation to the rainfall amounts of the preceding two days and two days following for analyses. Rainfall of >15 mm was defined as 'substantial' when the two previous days had <5 mm of rain and the two days following had <10 mm of rain.

Laboratory experiments

Captive studies were conducted to determine: i) the number of eggs consumed by individual tadpoles throughout development in the absence of competition with conspecifics, and ii) density-dependent effects on growth and survival of tadpoles.

Cannibalistic Capacity. To determine numbers of eggs consumed throughout development and if a diet consisting solely of conspecific eggs is sufficient to maintain tadpoles through development, I raised 16 tadpoles in isolation, providing each with conspecific eggs ad libitum as their exclusive food source. Single eggs were removed 1 hr after deposition from each of 16 different oviposition sites and placed in separate containers containing 50 ml of water. To approximate natural feeding intervals, I gave each of the hatched tadpoles eggs ad libitum every 8-9 d throughout development (50 eggs were given per feeding for first 3 weeks of development, 75 eggs per feeding thereafter). Any new tadpoles emerging from unconsumed eggs were removed prior to the next feeding event. Developmental time, size at metamorphosis, and number of eggs consumed at each feeding were recorded for each tadpole.

Density Experiment. To determine the effect of crowding on growth and survival, I raised tadpoles at various naturally-occurring densities. Because growth and

development may have a genetic component in tadpoles (e.g., *Pseudacris triseriata*, Travis 1981), full siblings were used in this experiment to minimize genetic variation among treatments. To obtain tadpoles, I took a single amplexed pair into the laboratory and placed them in water for egg deposition. Eight days later, the 558 tadpoles were individually and randomly assigned to six density treatments. Each treatment consisted of 50 ml of water with 1, 2, 5, 10, 25, or 50 tadpoles. There were six replicates of each treatment level. Tadpoles were fed ad libitum on a modified diet known to support normal development in other anurans (Harkey and Semlitsch 1988). This diet consisted of a mixture of Hartz guinea pig pellets, Gerber High Protein baby cereal, and trout chow (a substitute for fish flakes). Water was changed daily to remove uneaten food and to reduce any effects of excretory products in the small containers. Survival of tadpoles was recorded daily. Developmental time and size at metamorphosis were recorded for each individual.

Data Analysis

Throughout, means are reported ±1 SD (unless otherwise noted) and comparative tests are two-tailed. Alpha was set at 0.05.

For each of the predominate causes of tadpole mortality, the G-test of independence (which tests the goodness of fit of the observed frequencies to their expected frequencies, BIOMstat 1996) was used to determine if the cause of predation was independent of the nursery water volume. To lower the probability of type I errors, I adjusted the G-test statistics using Williams's correction (Sokal and Rohlf 1995). For this analysis, data were compiled from all clutches that suffered complete mortality by 64 d, or produced at least one froglet and/or had at least one tadpole survive to 64 d.

The Aspin-Welch-Satterthwaite (AWS) test, which is robust to unequal population variances when sample sizes are small and/or unequal (Toothaker and Miller 1996), was used in place of the two-independent-sample *t*-test. The AWS test statistics were computed using SAS statistical software (SAS 1997b). The Tukey Multiple Comparison Procedure (Tukey MCP) was used for all pairwise comparisons. Regressions were conducted in JMP IN statistical software (SAS 1997a). When separate independent regressions were used to analyze the data, overall significance was determined by Fisher's combined probability technique. This technique allows one to create an overall test for significance by combining the probabilities of several independent statistical tests (e.g., regressions within different females or within different clutches) that test the same hypothesis (Sokal and Rohlf 1995). To determine probabilities of Type II errors for statistical tests that had probabilities between 0.05 and 0.25 associated with the test statistics, I performed power analyses using the statistical program JMP IN (SAS 1997a). For each power analysis, alpha was 0.05 and the effect size was set to the square root of SSH/N where SSH was the sum of squares for the hypothesis and N equaled the total sample size. SYSTAT for Windows was used for all other analyses (SYSTAT 1997).

RESULTS

Females ovipositing in artificial sites did not differ from those using natural bromeliads with respect to either body size (mean SVL in cups = 70.6 ± 2.5 mm, $n = 15$ females; in bromeliads = 71.3 ± 2.4 mm, $n = 12$; AWS $t_{24.2} = 0.84$, $P = 0.41$) or mean inter-clutch interval (cups = 9.8 ± 2.3 d, $n = 15$ females; bromeliads = 10.4

± 1.9 d, $n = 12$; AWS $t_{25} = 0.72$, $P = 0.48$). Mean clutch size (including initial and secondary sub-clutches) was 602.84 ± 129.79 eggs per female cycle (range 354 - 856 eggs, $n = 15$ females). The number of eggs deposited increased with water volume in the three levels tested (one-way repeated measures ANOVA: volume effect, $F_{2,28} = 39.49$, $P < 0.001$, Tukey MCP: all volume comparisons, $P < 0.001$; Fig. 1), such that the density of eggs was essentially constant across volumes (2.24 ± 1.78 eggs/ml in the 25-ml cups, 2.46 ± 0.76 egg/ml in the 50-ml cups, and 2.01 ± 0.68 eggs/ml in the 100-ml cups; one-way repeated measures ANOVA, with female as repeated measure: volume effect, $F_{2,28} = 0.70$, $P = 0.50$). There was no difference between the number of eggs deposited in initial vs. secondary sub-clutches (paired-sample $t_{12} = 0.02$, $P = 0.99$). As in the artificial sites, number of eggs deposited in the bromeliads increased linearly with volume ($r^2 = 0.86$, $t_{12} = 8.50$, $P < 0.001$; Fig. 1).

Clutch size increased with inter-clutch interval (mean $r^2 = 0.27$, Fisher's combined probability $\chi^2_{10} = 20.79$, $P = 0.02$, $n = 5$ females that deposited at least five separate times in the artificial sites). Female SVL was also positively correlated with mean clutch size ($r^2 = 0.28$, $t_{13} = 2.28$, $P = 0.04$) and 8% more of the variation was explained when inter-clutch intervals were factored in ($r^2 = 0.36$, $t_{13} = 2.72$, $P = 0.02$, $n = 15$; Fig. 2).

Water volume and female site preference

Females were more likely to deposit eggs in sites containing greater amounts of water and their sub-broods were more successful in such sites. Most (88% ± 11%) of the 100-ml cups were used by the females for both initial and secondary clutches,

compared to 63% (± 27%) of the 50-ml cups and 48% (± 34%) of the 25-ml cups (one-way repeated measures ANOVA: $F_{2,38} = 15.7$, $P < 0.001$). Specifically, females preferred 100-ml cups over 50-ml ($P = 0.003$) and 25-ml cups ($P < 0.001$; Tukey MCP). Sub-brood failures occurred more often in the smaller volume cups than in the larger cups, with 12/13 in the 25-ml cups failing, 35/47 in the 50-ml cups, and 22/43 in the 100 ml cups ($G_{adj} = 10.28$, df = 2, $P = 0.006$; Fig.3).

The primary causes of mortality (to 64 days post-oviposition) varied among water volumes (Fig. 4). Carnivorous *Dendrobates ventrimaculatus* tadpoles, transported just after hatching by their parents and deposited singly into small pools of water, feed copiously on both conspecific and heterospecific tadpoles. These predators often were in the cups prior to *O. planiceps* oviposition so their presence was not necessarily a response to food availability (i.e., *O. planiceps* tadpoles). This predation was the leading cause of mortality in the 50-ml (40.4%) and 100-ml cups (30.2%), not differing between these two volumes ($G_{adj} = 2.46$, df = 2, $P = 0.29$). In 25-ml cups, *D. ventrimaculatus* tadpoles contributed to mortality in 20.0% of the sub-broods. On the other hand, the deposition of *O. planiceps* egg masses sporadically led to suffocation of dendrobatid tadpoles, with 2/22 (9%) dying in 25-ml, 11/52 (21%) in 50-ml, and 16/49 (33%) in 100-ml cups.

Tadpoles from initial sub-broods that were not fed nutritive eggs regularly began to exhibit visible signs of starvation 21 days (± 5 days) after the previous oviposition. None of the 23 such sub-broods survived to emergence. In the smallest nurseries (25-ml cups) starvation was the greatest cause of tadpole mortality: all tadpoles starved in 53.3% of these cups. Starvation was less often a factor of complete mortality in the 50-ml cups (23.4%) and the 100-ml cups (7.0%)

(G_{adj} = 13.58, df = 2, P = 0.001).

Low dissolved oxygen concentration (caused by egg deposition) apparently contributed to tadpole mortality in 20.0% of the 25-ml cups, but had little or no effect in the larger nurseries (0% in 50-ml cups; 4.7% in the 100-ml cups; G_{adj} = 7.65, df = 2, P = 0.02).

Another major cause of sub-clutch and/or sub-brood failure in all volumes was predation of frog embryos by dipteran larvae, which were found in 20.0% of 25-ml, 27.7% of 50-ml, and 20.9% of 100-ml cups (G_{adj} = 0.67, df = 2, P = 0.71).

Fewer than 15% of the sub-broods in each volume were taken directly by snakes (e.g., *Leptophis ahuetulla, Liophus reginae, Leptodiera annulata*) up to 64 days post-oviposition, but these predators probably had a disproportionate impact on tadpole mortality as their depredations were delayed until most tadpoles were near metamorphosis. At least six of the 16 study broods that experienced snake predation were pillaged after the 64th day of development.

Effects of oviposition and rainfall on oxygen levels

Although the dissolved oxygen concentrations in the catchments were highly variable (range = 0 – 8.3 ppm), the levels within catchments increased with rainfall (r^2 = 0.14, t_{69} = 3.33, P = 0.001, n = 71 consecutive days) and decreased following egg deposition (Fig. 5). Dissolved oxygen concentrations were highest on the day after rainfalls of more than 15 mm (day 1), in both the artificial oviposition sites (two-way ANOVA: day effect, $F_{5,60}$ = 29.55, P < 0.001; site effect, $F_{9,60}$ = 0.08, P = 1.0; interaction effect, $F_{45,60}$ = 2.48, P < 0.001; day 1 compared to all other days, P < 0.001, Tukey MCP; Fig. 6) and bromeliad leaf axils (two-way ANOVA: day effect, $F_{6,49}$ = 19.73, P = 0.001; site effect, $F_{6,49}$ = 0.02, P = 1.0; interaction effect,

$F_{36,49}=0.94$, $P=0.57$). Following egg deposition, however, dissolved oxygen levels decreased in the 100-ml cups, such that for each of the next two days after oviposition concentrations were lower than on the day of laying (two-way ANOVA: day effect, $F_{2,30}=176.48$, $P<0.001$; site effect, $F_{9,30}<0.001$, $P=1$; interaction effect, $F_{18,30}=4.34$, $P<0.001$; Fig. 7). Strong interaction effects partially reflect the variability in dissolved oxygen concentrations across oviposition sites as some sites maintained higher (or lower) oxygen levels than others.

Female response to rainfall

To determine if females selectively oviposit on particular days in response to rainfall, I calculated the number of females depositing eggs on the night of each 'substantial' rainfall (defined as >15 mm), the night before such rain events, and two nights immediately following rains. The mean rainfall amounts were 1.0 ± 1.4 mm for the day before the rain, 44.7 ± 24.3 mm the day of the rain, 2.0 ± 2.3 mm the day after the rain, and 5.1 ± 6.9 mm two days after the rain. Females clearly responded to rainfall (one-way ANOVA: $F_{3,40}=15.42$, $P<0.001$, $n=11$ four-day periods) and were more likely to oviposit on the day following substantial rainfall than on the day before ($P<0.001$), the day of ($P<0.001$), or two days after the rain ($P<0.001$, Tukey MCP; Fig. 8).

Because the females used in the previous analysis were not independent (i.e. 34 females deposited eggs for a total of 85 ovipositions within the 11 four-day periods), I tested whether individuals differed in their response to rainfall and found no differences among females (one-way ANOVA: $F_{33,202}=0.52$, $P=0.99$).

Tadpole survival

In the field, hatchling success differed among cup volumes whereas tadpole survival through metamorphosis was comparable across volumes (Table 1). Hatchling survival was inversely related to initial sub-clutch size for all three cup volumes (mean $r^2=0.46$, Fisher's combined probability $\chi^2_6=44.04$, $P<0.001$, Fig. 9) and tended to decline with volume (Table 1). The ratio of tadpoles surviving (through metamorphosis) to initial sub-clutch size (excluding sub-clutches that failed to produce any froglets) was similar in the 50-ml (0.045 ± 0.059, $n=12$) and 100-ml cups (0.067 ± 0.063, $n=17$; AWS $t_{24.9}=0.95$, $P=0.35$).

Although larger females deposited more eggs than smaller females throughout tadpole development, maternal size predicted neither number of froglets produced ($r^2=0.01$, $t_9=0.26$, $P=0.80$, Fig. 10) nor size at metamorphosis ($r^2=0.06$, $t_9=-0.75$, $P=0.47$).

Egg and larval cannibalism

Tadpoles from previously deposited sub-clutches were still present on 91% (413/454) of the occasions when females returned to their oviposition sites.. In the remaining 41 cases, the newly deposited eggs became initial sub-clutches and produced an average of 4.2 ± 3.4 froglets 50% of the time.

In catchments containing tadpoles, most eggs were consumed by their older siblings within hours of oviposition. Only seven of 424 (1.6%) secondary sub-clutches (i.e., sub-clutches deposited in cups already containing one or more tadpoles) produced any froglets and five of these secondary sub-clutches were deposited in catchments that each contained just a single tadpole from previously depos-

ited clutches. In the oviposition sites for which complete data are available from deposition to metamorphosis, the survival of tadpoles that began as secondary sub-clutches (all cup volumes pooled) was virtually negligible (0.03% ± 0.13%, $n = 422$ secondary sub-clutches).

The number of eggs consumed by tadpoles through development when fed ad libitum in the laboratory was well within the range of food naturally available (i.e., eggs and conspecific tadpoles) per successfully metamorphosed froglet that developed in the artificial oviposition sites. Specifically, in the laboratory feeding experiment the mean number of eggs consumed by tadpoles through development to metamorphosis was 97.2 ± 23.3 eggs ($n = 16$ tadpoles): in the field, an average metamorphosed froglet ate 126.7 ± 31.8 eggs (range 84.2 - 166.0 eggs, $n = 5$) in the 50-ml cups and a very similar 128.7 ± 29.8 eggs (range 84.3 - 171.5 eggs, $n = 10$) in the 100-ml cups. The number of nutritive egg depositions depended upon the maximum developmental time for the tadpoles and ranged from 4 to 12 depositions per initial sub-clutch in the 50-ml cups (mean = 6.0 ± 2.4, $n = 12$) and 3 to 19 in the 100-ml cups (mean = 8.7 ± 5.2, $n = 17$). More nutritive eggs were delivered to the larger (100-ml) cups ($mean_{50ml} = 911.0 \pm 429.6$ eggs, $mean_{100ml} = 1731.6 \pm 897.0$ eggs; AWS $t_{24.3} = 3.28$, $P = 0.003$), where twice as many froglets emerged (10.5 ± 9.2 vs. 4.6 ± 4.5) (AWS $t_{24.6} = 2.29$, $P = 0.03$).

In the laboratory, the number of eggs consumed per tadpole per feeding was low (< 5 eggs) during the first week of development, then steadily increased from day 10 to day 30 of development. At peak consumption (one feeding event at 25 to 30 d), individual tadpoles ate an average of 46.1 ± 11.6 eggs (range 24 - 65) during that feeding interval. After day 30 the number of eggs consumed per tad-

pole decreased as metamorphosis neared ($n = 16$, Fig. 11).

In the field, larval cannibalism began during the third week of development, coinciding with the increased egg consumption exhibited by the laboratory tadpoles. Survivorship was analyzed for sub-broods from the 25, 50, and 100-ml cups in which no extrinsic losses of tadpoles (e.g., predation) occurred (Fig. 12). Even though there was no statistical difference found in survivorship across the three volumes, a significant interaction effect (two-way repeated measures ANOVA: volume effect, $F_{2,192} = 2.58$, $P = 0.09$, power = 0.48; day effect, $F_{12,192} = 17.52$, $P < 0.001$; interaction effect, $F_{24,192} = 2.62$, $P < 0.001$) suggested that the volumes had differing effects on survival, most notably soon after hatching. Survival of tadpoles in the 50-ml cups dropped initially by 37.5% (± 31.7%), leveled off slightly, then began to decrease markedly during the third week of development, presumably due to sibling cannibalism, before steadying at about 9% (± 4%) of the original brood size as tadpoles began to metamorphose ($n = 5$ clutches). The survival curves of tadpoles in 25-ml cups ($n = 2$ clutches) were roughly similar to those in the 50-ml cups. By contrast, survival in the 100-ml cups ($n = 12$) showed a sharper initial loss of 61.9% (± 28.8) at hatching, then a steady, but more gradual, decline thereafter. Tadpole densities leveled off by the seventh week of development in both the 50-ml (0.3 ± 0.07 tadpoles/ml) and 100-ml cups (0.25 ± 0.14 tadpoles/ml) and were similar in the two volumes (AWS $t_{14.2} = 0.82$, $P = 0.43$).

Among the laboratory density experimental treatments, there was an increasing proportional loss of tadpoles with increasing density (Kruskal-Wallis one-way ANOVA, $\chi^2_6 = 21.86$, $P < 0.001$). In the lower density treatments (1 and 2 tadpoles per 50 ml water), all tadpoles reached metamorphosis. Average mortality was

8.0% or less in the 5, 10, and 25 tadpoles per 50 ml water treatments, and increased to 19.7 ± 3.9% in the highest concentration (50 tadpoles/50 ml water). This treatment matched the median density of tadpoles observed in the 50-ml field cups at 8 d post-oviposition (median ±1 median absolute deviation = 52 ± 33.4 tadpoles, n = 35 clutches).

Developmental time of tadpoles and size at metamorphosis

Density of tadpoles, not volume of oviposition site, determined developmental time (the span between oviposition and emergence of froglets). Minimum developmental times (to first froglets) were comparable in the 50-ml cups (48. 7 ± 12.1 d, $n = 15$ cups) and 100-ml cups (46.9 ± 12.5 d, $n = 24$ cups; AWS $t_{30.7} = -0.43$, $P = 0.67$), as were the mean developmental times for these two volumes ($mean_{50ml} = 53.9 \pm 12.7$ d, $mean_{100ml} = 53.5 \pm 13.4$ d; AWS $t_{24.6} = -0.08$, $P = 0.94$, $n_{50ml} = 12$, $n_{100ml} = 16$). Because only one of the 25-ml cups produced froglets, that treatment was excluded from analysis. In the laboratory, where I controlled for volume of water while varying tadpole density, developmental times were longer in the higher density treatments, both for minimum (one-way ANOVA: $F_{5,30} = 34.82$, $P < 0.001$) and mean developmental times (one-way ANOVA: $F_{5,30} = 437.32$, $P < 0.001$; Fig. 13a). Tadpoles developing in higher densities also varied more in their developmental times (one-way ANOVA: $F_{4,25} = 93.63$, $P < 0.001$; Fig. 13b).

Froglets emerging from cup sites in the field were similar in size to those emerging from bromeliads. Size at emergence for froglets that developed in a sample of five bromeliads (mean SVL = 11.8 ± 0.8 mm, range = 10.8 - 12.7 mm) was matched by the overall mean for all artificial sites (pooled mean SVL = 11.6 ± 0.9

mm, range 9.9 - 13.6 mm, $n=48$ sites; AWS $t_5=0.54$, $P=0.61$). Froglets emerging from 50-ml cups (mean SVL = 11.6 mm ± 0.7 mm, $n=21$) were the same size as those from 100-ml cups (11.6 mm ± 0.9 mm, $n=27$; AWS $t_{46}=-0.09$, $P=0.93$). By contrast, tadpole density in the laboratory experimental treatments had a strong negative effect on froglet size (one-way ANOVA: $F_{5,30}=6.55$, $P<0.001$; Fig. 14a). The variance in froglet size among the experimental treatments did not differ across initial tadpole densities (one-way ANOVA: $F_{4,24}=0.12$, $P=0.98$; Fig. 14b).

In general, individuals that spent more time in the tadpole phase were larger upon completing metamorphosis. In ten of the 11 cups that produced at least ten froglets (50 ml, $n=2$ clutches; 100 ml, $n=9$ clutches), developmental time was related to size at metamorphosis in a positive and linear fashion (mean $r^2=0.59$, Fisher's combined probability $\chi^2_{22}=149.74$, $P<0.001$). This relationship even held across sub-broods, despite the varying conditions (e.g., size of initial sub-clutch, density, amount of food provided during development, cannibalism, predation, etc.) experienced in the different sites ($r^2=0.40$, $t_{181}=9.97$, $P<0.001$, Fig. 15).

Tadpoles raised in the laboratory, both when raised singly and fed eggs and when raised in density experiments, showed somewhat weaker relationships between developmental time and metamorph size, but it is not clear whether this was due to the artificial conditions (e.g., ad libitum food combined with high density), to low statistical power from modest sample sizes (power for the null results ranged from 0.10 to 0.37), or to both (see Appendix I for full details).

DISCUSSION

The reproductive mode of laughing frogs differs from most other anurans whose tadpoles practice obligatory oophagy. Typically, species that provide nutritive eggs for developing larvae have low fecundity (Salthe and Duellman 1973) or low hatching rate (Lannoo et al. 1987, Jungfer 1996, Kam et al. 1996), and the tadpoles are fed unfertilized, so-called "trophic eggs" (Weygoldt 1980, Brust 1993, Lannoo et al. 1987, Jungfer 1996, Kam et al. 1996) that necessarily have no potential for developing into independent offspring themselves. Among bromeliad-breeders, *O. planiceps* exhibits an exceptional combination of three features: (i) relatively high fecundity, (ii) high hatching success, and (iii) the provisioning of fertilized nutritive eggs to tadpoles. *O. planiceps* females are able to assess the water volume at oviposition sites and deposit eggs accordingly, depositing on average about 2 eggs per ml of water, regardless of nursery volume. On average, an initial clutch of 602 eggs is deposited, divided as sub-clutches among several water-filled leaf axils, and from these initial clutches an average of only 9 froglets emerge (1.5% survival). Even though larger females deposit more eggs, they may not produce more froglets. Also, their tadpoles at metamorphosis are no larger than those of smaller females. These results, which suggest that female size and fecundity may be poor predictors of reproductive success, reflect the stochastic nature of the oviposition sites (see Fincke and Hadrys, 2001 for similar findings in the damselfly, *Megaloprepus coerulatus*).

Although *O. planiceps* females adjust sub-clutch size to the volume of water contained within each catchment, the nurseries do not afford enough space for all eggs from initial clutches to survive to metamorphosis. *O. planiceps* tadpoles are

cannibalistic and the nutrients needed to sustain these tadpoles through metamorphosis are available in the form of nursery-mates and conspecific eggs. Throughout larval development, tadpoles feed on one another and eat younger full or half-siblings supplied by their mother in the form of fertilized eggs. The production of excess eggs in the initial clutch and the deposition of additional eggs throughout tadpole development allows mothers to provision their offspring with an energy-rich resource. On average, only three out of every 10,000 secondary-clutch eggs survived through metamorphosis. Over the course of development, each emerging froglet consumes an average of 128 siblings. This overproduction of offspring is essential for tadpole survival in the food-limited microhabitats of water-filled leaf axils.

The stochastic abiotic characteristics of axil catchments present another challenge for species ovipositing in these sites. Amphibian embryos respire aerobically, thus require oxygen for growth and development (Adolph 1979). In addition, respiration increases with age, thereby increasing the rate of oxygen consumption as embryos develop (Atlas 1938, Moore 1940). Against this metabolic need, the small pools contained within bromeliad axils are often hypoxic (Laessle 1961). Dissolved oxygen concentration of the water is extremely variable, both among and within bromeliads, fluctuating as functions of bromeliad morphology, location, rainfall, wind, debris accumulation, and the aquatic community composition (Laessle 1961).

One solution to the variability of oxygen levels within arboreal habitats is for anuran taxa to oviposit out of the water, either on the inner walls of tree holes (e.g., *Anotheca spinosa*, Jungfer 1996, *Chirixalus eiffingeri*, Kam et al. 1996), on vegetation just above water (e.g., *Agalychnis calcarifer*, Marquis et al. 1986, Donnelly et al. 1987), or in terrestrial nests (e.g., *Dendrobates pumilio*, Weygoldt

1980). Although these patterns may have arisen primarily for aquatic-predator avoidance (Caldwell 1994), eggs deposited out of water also have a better chance of being well-oxygenated, resulting in faster rates of embryonic development (Salthe and Duellman 1973, Salthe and Mecham 1974, Bradford 1990, Seymour and Roberts 1995, Kam et al. 1998).

An alternative strategy is to deposit eggs in the water of arboreal sites selectively, choosing temporal windows when dissolved oxygen levels is elevated. Rainfall replenishes the dissolved oxygen concentration of water held within bromeliad leaf axils, potentially compensating for the oxygen-depressing effects from embryo respiration. *O. planiceps* females adjust their inter-clutch intervals in response to rainfall by depositing most frequently on nights just after substantial rainfalls.

Most *O. planiceps* eggs are deposited as a single-layer surface film, a pattern that further increases the oxygen concentration surrounding the eggs (Moore 1940, Zweifel, 1964), though some eggs become submerged, depending on the surface/volume ratio of the water held within the leaf axils. When submerged, anuran eggs experience retarded development, and often die due to hypoxic conditions (Moore 1940, Pyburn 1970, Kluge 1981, Kam et al. 1998). Females typically do not deposit eggs during heavy rainfall, perhaps because the raindrops can cause newly deposited eggs to lose their surface adhesion and sink (Kluge 1981).

The embryos of *O. planiceps* begin to hatch relatively quickly (ca 36 hours after oviposition) compared to the developmental rates of other anurans (reviewed in Duellman and Trueb 1986), which may be in response to the increasingly hypoxic condition of the nursery. As the embryos develop, the dissolved oxygen concentration decreases at accelerating rates. Larger sub-clutches will quickly exhaust the available dissolved oxygen, thereby decreasing hatching success. Poulin and Fitz-

Gerald (1989) reported a similar negative relationship between clutch size and hatching success in three-spined sticklebacks, which was attributed to the increased rate of oxygen consumption of the larger clutches (Reebs et al. 1984). In some anurans, once the embryos have completed sufficient development, low oxygen levels become the primary proximate stimulus to hatching (e.g., *Pseudophryne bibronii*, Bradford and Seymour 1988; *Crinia georgiana*, Petranka et al. 1982; Seymour and Roberts 1995). *O. planiceps* hatchlings retain external gills and suspend from the water surface immediately following hatching, presumably to acquire oxygen.

Accelerated development of *O. planiceps* embryos may have evolved in response to the high mortality rate of embryos. By hatching early, not only do the tadpoles avoid predation by oophagous nursery-mates in the embryonic stage, but they may also have the opportunity to eat early in their development, potentially accelerating their growth rate (Eickwort 1973, Polis 1981, Lannoo et al. 1987, Baur 1992, Crump 1992). Due to the enhanced growth rate of those individuals that consume eggs soon after hatching, a size hierarchy among tadpoles quickly emerges that may, in turn, promote further cannibalism of larval nursery-mates (Polis 1981, Elgar and Crespi 1992).

The benefits of sibling cannibalism are two-fold: the cannibalistic larvae obtain nourishment when food resources are low and competition for limited resources within the nursery is relaxed (Fox 1975, Crump 1992). The timing of increased sib-cannibalism in the 50-ml oviposition sites coincided with the peak of egg consumption demonstrated by the laboratory tadpoles fed eggs throughout development. In the 100-ml oviposition sites, onset of larval cannibalism was less apparent; rather tadpole survivorship declined steadily throughout development. This difference may

be due to differences in hatching success in 50-ml vs. 100-ml cups. Hatching success was inversely related to the number of eggs deposited within an oviposition site, with proportionately fewer tadpoles hatching from larger sub-clutches. Because more eggs were typically deposited in the larger volume sites, hatching success tended to be lower in those sites. Thus, there were fewer tadpoles and more non-larval food (unhatched eggs) available per tadpole during the first week of development in the larger nurseries. Because females deposit more eggs in the larger volume oviposition sites, tadpoles in those sites continued to be provided with more eggs per tadpole throughout development. These lines of evidence suggest that larval cannibalism is facultatively based in part on egg supplies.

Across taxa, cannibalism most often occurs when populations are food-stressed (Fox 1975, Polis 1981, Elgar and Crespi 1992), but it can also occur as a direct response to overcrowding, even when resources are abundant (Fox 1975, Fincke 1994). There are several indications that high density is a more important proximate trigger than lack of food for sibling cannibalism in *O. planiceps*. Even when fed steadily by the mother throughout development, tadpoles tend to cannibalize nursery-mates. Conversely, several unfed broods at low densities were not cannibalistic even though the tadpoles were visibly starving (*personal observation*). And when tadpoles were fed ad libitum in the laboratory, mortality was substantially greater in the high-density treatments, indicating that limited food resources are not required for cannibalism to occur. Gromko et al. (1973) suggested that the higher mortality rates found in higher density experimental amphibian populations may be due to a crowding effect mediated by the effective population size. Although I observed larval cannibalism, tadpole mortality in the high density treatment may have been the result of a crowding effect and not cannibalism per se. Thus,

the proximate cause of larval cannibalism appears to be density-dependent, regardless of food availability.

Due to female assessment of water volume when depositing eggs, clutch-size dependent hatching failure, and tadpole density-dependent mortality, tadpole densities were comparable across the volumes throughout development. The minimum and mean developmental time and size of newly transformed froglets were similar in the two larger volumes tested, further indicating that in this species, density is an important factor in larval survival to metamorphosis.

Density affected developmental time and size of newly transformed froglets. Tadpoles in the higher density treatments had longer minimum and mean developmental times, and showed greater variance in developmental times than tadpoles in the low density treatments. The mean froglet size at emergence decreased with greater densities, but variability remained the same across densities. Similar relationships between density and both larval period and size at emergence in other amphibians were reviewed by Wilbur (1980). Due either to individual genetic differences or chance differences in physiology and/or feeding opportunities, some tadpoles experience rapid initial growth and may inhibit the growth of cohorts, through either interference competition or hormonal interactions (Wilbur and Collins 1973, Gromko et al. 1973). These faster growing tadpoles under high density conditions may then metamorphose at the minimum size possible to escape the density stress; their metamorphosis, in turn, lowers the effective micro-population density and releases the remaining tadpoles from some of the food competition, which has the effect of allowing them better opportunity to grow (Wilbur and Collins 1973). Because *O. planiceps* females adjust the number of eggs deposited to the volume of water at each site, regardless of the number of tadpoles present, food becomes

more abundant for the remaining tadpoles over time. When conditions are favorable, tadpoles should consume more nutrients, develop more slowly, and metamorphose at larger sizes (Wilbur and Collins 1973). As expected, under semi-natural conditions, *O. planiceps* froglets that had spent greater amounts of time as tadpoles were larger upon completing metamorphosis.

Female laughing frogs selectively oviposited in the larger volume sites, even though they produced the same relative number of tadpoles per unit effort (i.e., on average twice as many eggs were deposited and twice as many froglets emerged from the 100 ml cups vs. the 50 ml cups). Many anuran species exhibit site preferences, which Crump (1991) suggests is adaptive because females selecting less favorable habitats will, on average, leave fewer descendants. Not only were *O. planiceps* females more likely to oviposit in the larger volume sites, but sub-broods were more successful in those sites. The cause of tadpole mortality differed across nursery volumes. In the smaller volumes, most mortality was due to starvation (because females deposited nutritive eggs less frequently in the smaller volumes) and lack of oxygen that occurred following egg deposition. In the larger sites, tadpole mortality was most often caused by heterospecific tadpole predation. Females may oviposit preferentially in larger volume sites because of the superior surface-to-volume ratios, thus potentially higher dissolved oxygen concentrations. Although dry periods are uncommon, when they do occur the larger volumes may be more likely to retain water.

Phytotelme-breeding has evolved independently several times in various anuran lineages, resulting in diverse behavioral, morphological, and physiological adaptations in response to these nutrient-poor environments. In some bromeliad-breeding species, non-feeding tadpoles complete their development solely on the

nourishment provided by a large yolk; in others, tadpoles feed on trophic (unfertilized) eggs deposited by the mother. *O. planiceps* exhibits a reproductive mode in which tadpoles are cannibalistic towards nursery mates and are provisioned with fertilized nutritive eggs throughout development.

Why do laughing frogs provide fertilized eggs as food? Thompson (1992) suggested that *Osteopilus brunneus* females may deposit fertilized nutritive eggs early in tadpole development to avoid contamination of the oviposition site. Unfertilized eggs spoil rapidly and if not consumed will promote increased fungal and bacterial growth making the site unsuitable for weeks. For *O. planiceps*, the deposition of fertilized eggs not only reduces the risk of contamination, but also is advantageous to the parents because tadpole mortality is extremely high in this species. While females appear to have some ability to choose oviposition sites with optimal physical conditions for larval development, mortality is unpredictable. Fully 67% of the initial sub-clutches studied failed to produce any froglets. When a female returns to feed her tadpoles there is a nontrivial probability that some or all of her sub-broods have already succumbed to predation. Although 99.99% of all eggs in secondary sub-clutches are consumed by older siblings when the latter are still alive, those same eggs become potentially viable clutches on those occasions when the initial sub-brood happens to have been eliminated by stochastic events.

The fate of *O. planiceps* eggs is thus determined almost entirely by the presence vs. absence of older siblings. This species breeds year-round and when a sub-brood is eliminated or when all froglets emerge, a new sub-clutch is produced. A sub-clutch that would have fallen victim to oophagy had members of the initial sub-brood persisted instead becomes essentially an initial sub-clutch itself. Under natural conditions, most sub-clutches (91%) from any given oviposition may function as

food for older siblings (and have a 1.65% chance of producing froglets), while other sub-clutches from the same clutch and deposited in sites devoid of older siblings may function as initial sub-clutches, having a 33.0% chance of producing froglets.

Through initial over-production (i.e., deposition of more eggs than can survive through metamorphosis in the limited space of the oviposition sites), females provide a subset of the initial clutch with essential nutrients, while simultaneously creating replacement offspring in the event of loss. The subset of tadpoles from the initial clutch that survive through metamorphosis can be considered the 'core brood,' as defined by Mock and Forbes (1995). The remaining tadpoles from the initial clutch and all the fertilized nutritive eggs deposited by the female throughout tadpole development can then be considered to be 'marginal' offspring, that is, relatively expendable (Mock and Forbes 1995). Marginal offspring serve two roles in *O. planiceps*: sib-facilitation and insurance. When cannibalism occurs, the victims enhance the growth and development of their siblings, which are typically full sibs (Haugen Chapter 2). Over-production in the initial brood, along with the deposition of fertilized nutritive eggs, also functions as insurance against brood failure, by providing potential back-up offspring capable of taking over a survivor's slot should such an opening appear. Although the reproductive value of secondary sub-clutches is very low when older siblings are present (only 0.03% of larvae from nutritive eggs survive to metamorphosis), the insurance value is substantial due to the high occurrence of total brood loss. By taking the extra trouble to arrange for the continually deposited secondary egg-masses to be fertilized, the *O. planiceps* female is assured that her investments of energy and nutrients for producing ova are not wasted, but can contribute to fitness either in the form of viable new offspring or as food for a more advanced brood.

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APPENDIX I

No relationship was found between developmental time and size at metamorphosis ($r^2=0.07$, $P=0.32$; $n=16$; power $=0.16$) or between number of eggs consumed and metamorph size ($r^2=0.06$, $P=0.37$; $n=16$; power $=0.14$) for the laboratory tadpoles that were housed separately and fed eggs throughout development. In the density experiment, the treatment level of 25 tadpoles/50 ml water was the only one to show a significant positive relationship between developmental time and size at metamorphosis ($r^2=0.28$, $P<0.001$; $n=138$). One replicate in the 50 tadpoles/50 ml water also showed a significant positive relationship ($r^2=0.13$, $P=0.03$, $n=38$), however, the other four replicates did not, resulting in a nonsignificant correlation for the treatment level (50 tadpoles: $r^2=0.009$, $P=0.14$, $n=241$; power $=0.31$). The treatment level of five tadpoles/50 ml water showed an inverse relationship with metamorph size decreasing with developmental time ($r^2=-0.18$, $P=0.03$, $n=28$), and the one, two, and ten tadpoles/50 ml water treatments all showed no relationship (1 tadpole: $r^2=-0.15$, $P=0.45$, $n=6$, power $=0.1$; 2 tadpoles: $r^2=0.014$, $P=0.73$, $n=11$, power $=0.06$; 10 tadpoles: $r^2=0.05$, $P=0.10$, $n=58$, power $=0.37$).

Table 1. Mean percentage of hatchlings and froglets to initial sub-clutch size among the three volumes tested in the field. Hatchling success decreased with volume (one-way repeated measures ANOVA with female as repeated measure: volume effect $F_{2,24}=5.16$, $P=0.01$). Analyses limited to the 13 females that oviposited in all three volumes.

	hatchling success	survival through metamorphosis
25 ml	59.2 ± 22.2	1.2 ± 3.5
50 ml	45.9 ± 28.0	3.0 ± 6.5
100 ml	31.9 ± 26.6	2.0 ± 2.7

CAPTIONS

Figure 1. Mean number of eggs (+1 SD) deposited in artificial oviposition sites ($n = 15$ females) and bromeliads (25 ml, $n = 3$; 50 ml, $n = 5$; 100 ml, $n = 4$). In the artificial sites, the number of eggs deposited differed across volumes (two-way RM ANOVA, $P < 0.001$; Tukey MCP, $P < 0.001$ for all 3 volume comparisons).

Figure 2. Relationship between female SVL and number of eggs deposited per day as calculated by the mean clutch size/mean inter-clutch interval ($r^2 = 0.36$, $P = 0.02$; $n = 15$ females).

Figure 3. Mean percentage of sites (+95% CI) females used for oviposition and the percent initial sub-clutch failure for the different cup volumes ($n = 20$ females). Females preferred to oviposit in the higher volume sites (one-way RM ANOVA, $P < 0.001$) and percent clutch failure decreased with increase in cup volume.

Figure 4. Causes of egg and tadpole mortality as a function of catchment volume. The percentage of occurrence of mortality due to predation, low dissolved oxygen concentrations, and starvation of tadpoles through 64 days of development.

Figure 5. Three representative catchments (a: artificial; b and c: bromeliads) illustrating the change in dissolved oxygen concentration with rainfall (represented by vertical bars) and egg deposition over the same 3-week period (14 March to 3 April 1996). Two of the catchments (a and b) contained tadpoles. The arrows indicate days of oviposition.

Figure 6. The effect of rainfall on oxygen levels in artificial oviposition sites. The line represents the average change in oxygen levels (±95% CI) in 100-ml cups ($n = 10$ sites, 2 cups per site) two days prior to a rainfall amount of more than 15 mm of rain ($n = 5$), on the day of the rainfall (day 0), and for 3 days after the rainfall. The bars represent the average amount of rainfall (+95% CI) for those days.

Figure 7. Change in dissolved oxygen with egg deposition. The plot shows the average change (±95% CI) in dissolved oxygen concentration (ppm) before and after egg deposition (day 0) in 20, 100-ml oviposition cups, two cups per site ($n = 10$).

Figure 8. Female oviposition response to rainfall. The line connects the mean proportion of females (±95% CI) at the study site that oviposited on the night of substantial rainfall (day 0), the night before the rainfall (day -1), and two nights following the rainfall (days 1 and 2). These data were obtained from 11 4-day periods and include a total of 85 egg deposition events by 34 females ($n = 11$). Mean rainfall (+95% CI) is represented by the bars.

Figure 9. Regressions of hatchling survival at 4 days post-oviposition to number deposited eggs in (a) 25-ml ($n = 18$ sub-clutches), (b) 50-ml ($n = 31$ sub-clutches), and (c) 100-ml ($n = 34$ sub-clutches) ovipositions sites. Proportion of tadpoles alive at 4 days post-oviposition was inversely related to number of eggs deposited in all three volumes. Mortality in these clutches was not due to egg or tadpole predation.

Figure 10. Number of froglets produced per volume of water retained within the nurseries as a function of maternal SVL. No relationship was found between female size and number of froglets produced per sub-clutch ($r^2 = 0.01$, $n = 11$ females).

Figure 11. Mean number of eggs (±1 SD) eaten throughout development by tadpoles in lab ($n = 16$).

Figure 12. Mean percent tadpole survival (±1 SD) in (a) 25-ml ($n = 2$), (b) 50-ml ($n = 5$), and (c) 100 ml ($n = 12$) oviposition sites through 64 days of development. Loss of tadpoles in these sites was not due to egg or tadpole predation (other than cannibalism).

Figure 13. The upper figure (a) represents the relationship between tadpole density and mean time of development to metamorphosis. The lower figure (b) shows the differences in the mean variance of developmental time within the different density treatments. There were six replicates of each treatment level; all tadpoles were full siblings. Error bars = +95% CI.

Figure 14. The upper figure (a) represents the relationship between tadpole density and mean size at metamorphosis. The lower figure (b) shows the mean variance in size at metamorphosis within the different density treatments. Details as in Figure 12.

Figure 15. Size at metamorphosis increased with developmental time in the 50-ml and 100-ml oviposition sites ($r^2 = 0.40$). Data were pooled from all sub-clutches that produced at least 10 froglets (11 sub-clutches, $n = 183$ froglets).

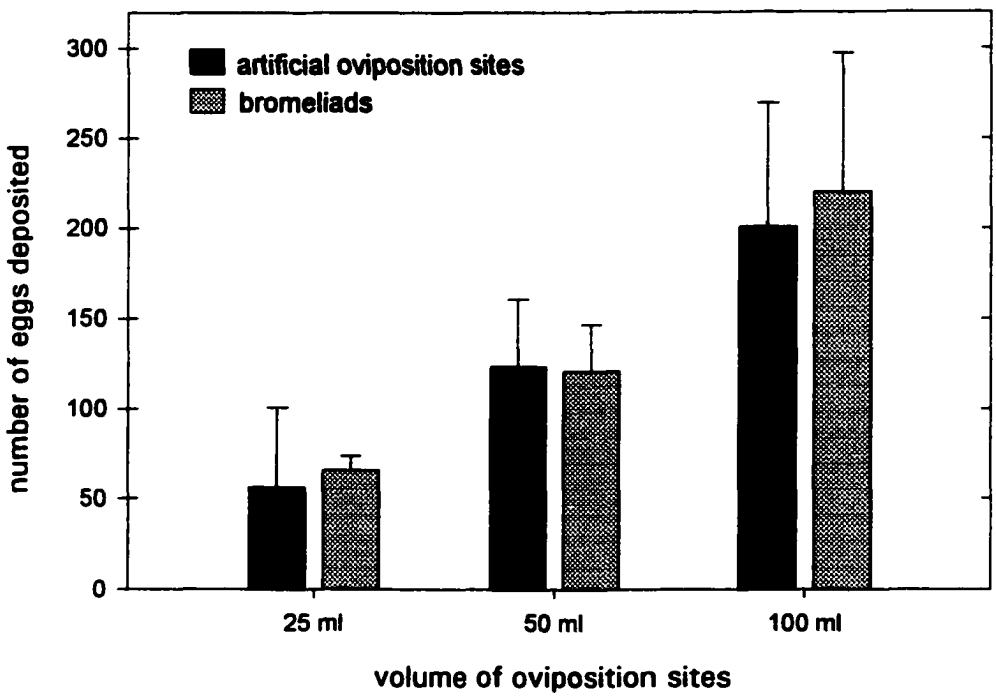

artificial oviposition sites
bromeliads
number of eggs deposited
300
250
200
150
100
50
0
25 ml
50 ml
100 ml
volume of oviposition sites

FIGURE 1

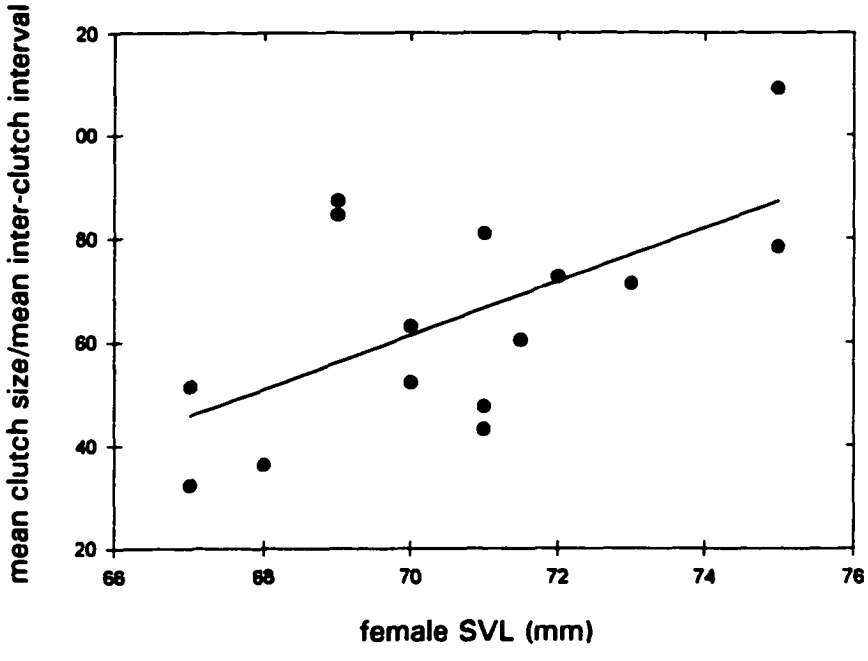

mean clutch size/mean inter-clutch interval
20
00
80
60
40
20
66
68
70
72
74
76
female SVL (mm)

FIGURE 2

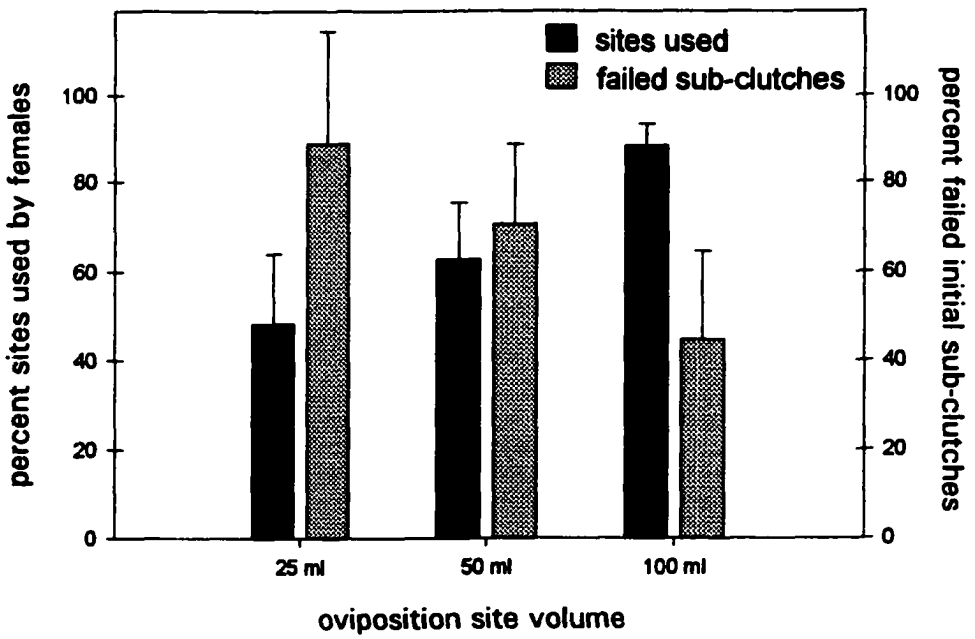

sites used
failed sub-clutches
percent sites used by females
percent failed initial sub-clutches
0
20
40
60
80
100
25 ml
50 ml
100 ml
oviposition site volume

FIGURE 3

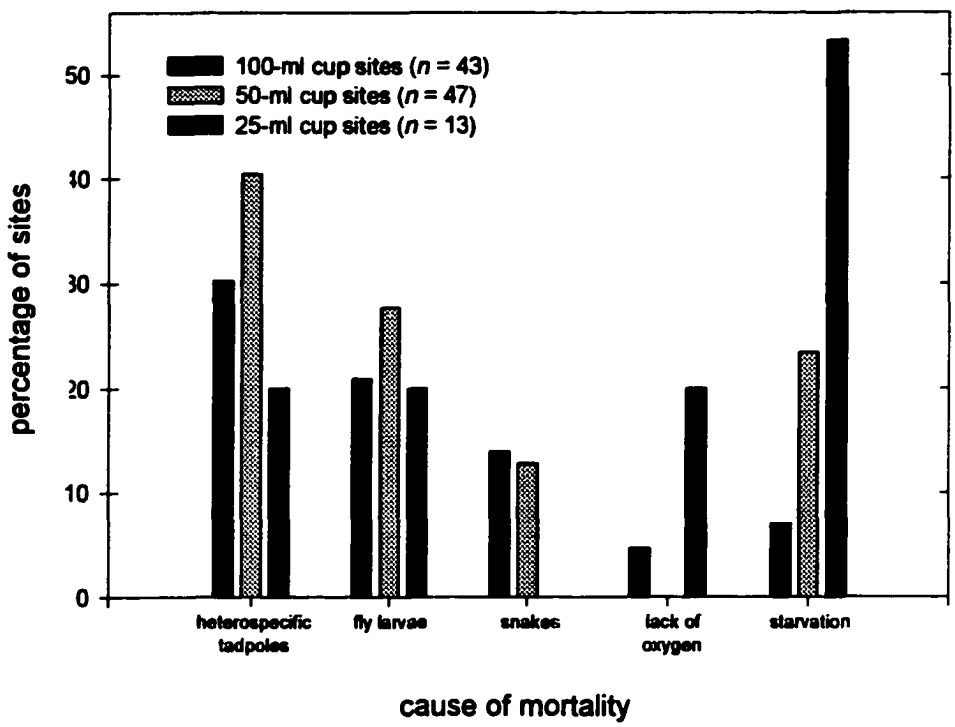

100-ml cup sites (n = 43)
50-ml cup sites (n = 47)
25-ml cup sites (n = 13)
percentage of sites
50
40
30
20
10
0
heterospecific tadpoles
fly larvae
snakes
lack of oxygen
starvation
cause of mortality

FIGURE 4

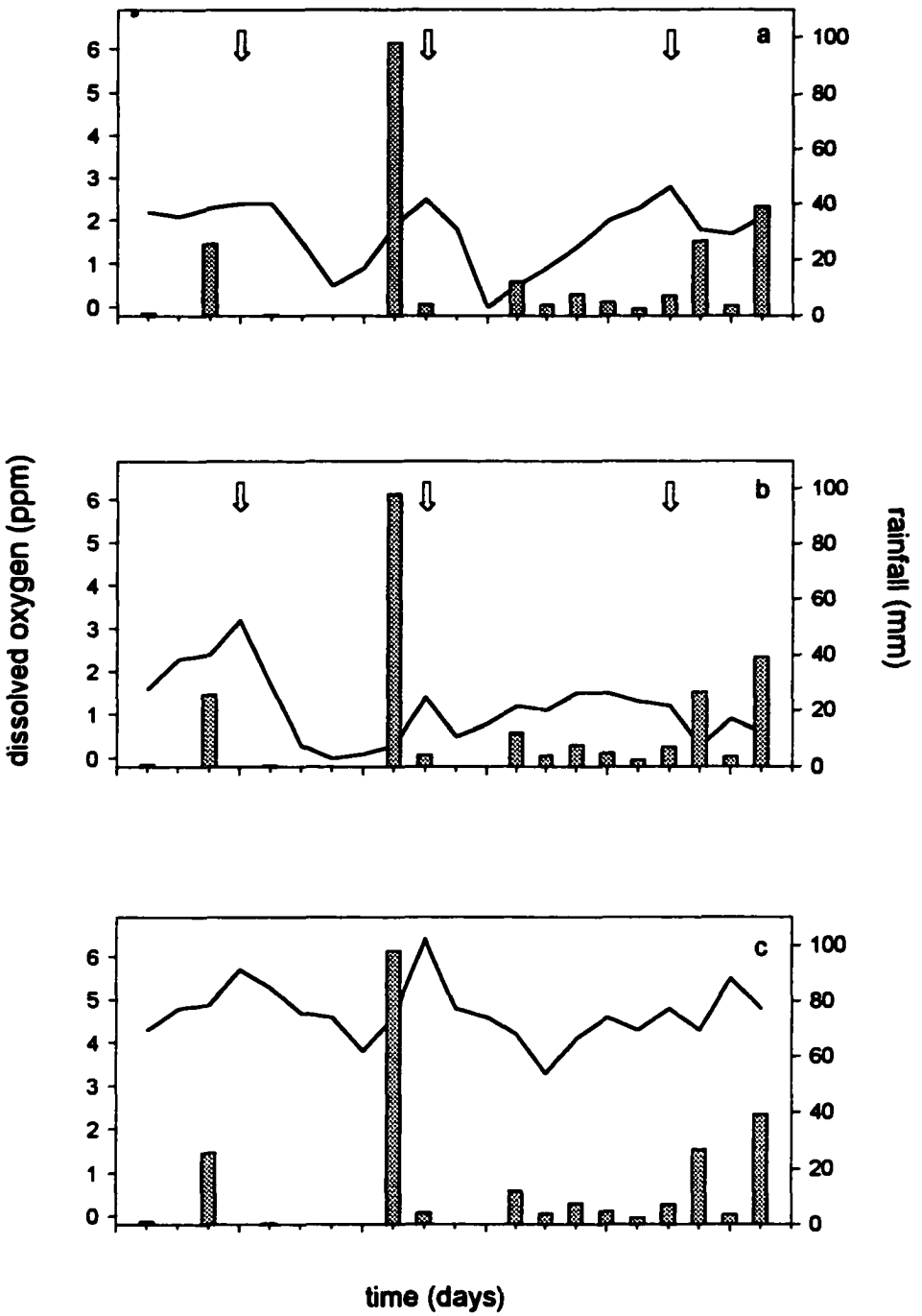

a
b
c
dissolved oxygen (ppm)
rainfall (mm)
time (days)
0
1
2
3
4
5
6
0
20
40
60
80
100

FIGURE 5

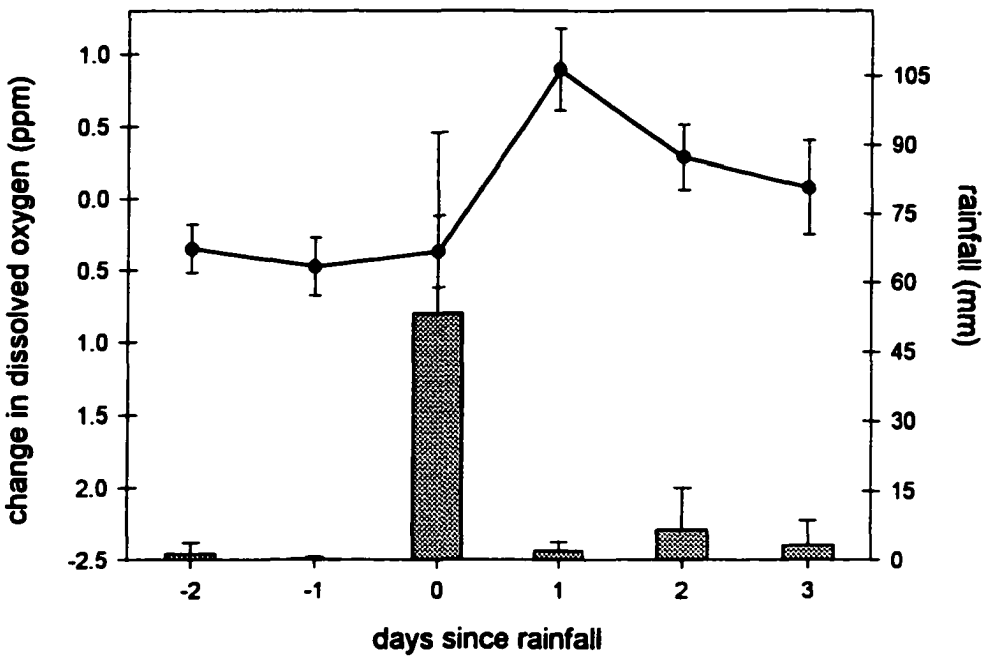

change in dissolved oxygen (ppm)
1.0
0.5
0.0
0.5
1.0
1.5
2.0
-2.5
rainfall (mm)
105
90
75
60
45
30
15
0
-2
-1
0
1
2
3
days since rainfall

FIGURE 6

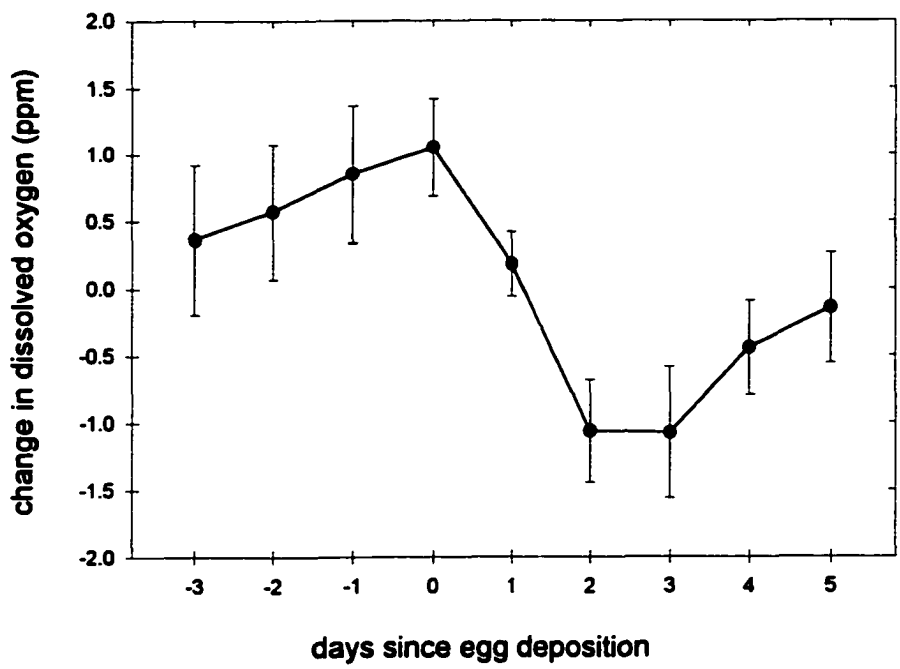

change in dissolved oxygen (ppm)
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
-3
-2
-1
0
1
2
3
4
5
days since egg deposition

FIGURE 7

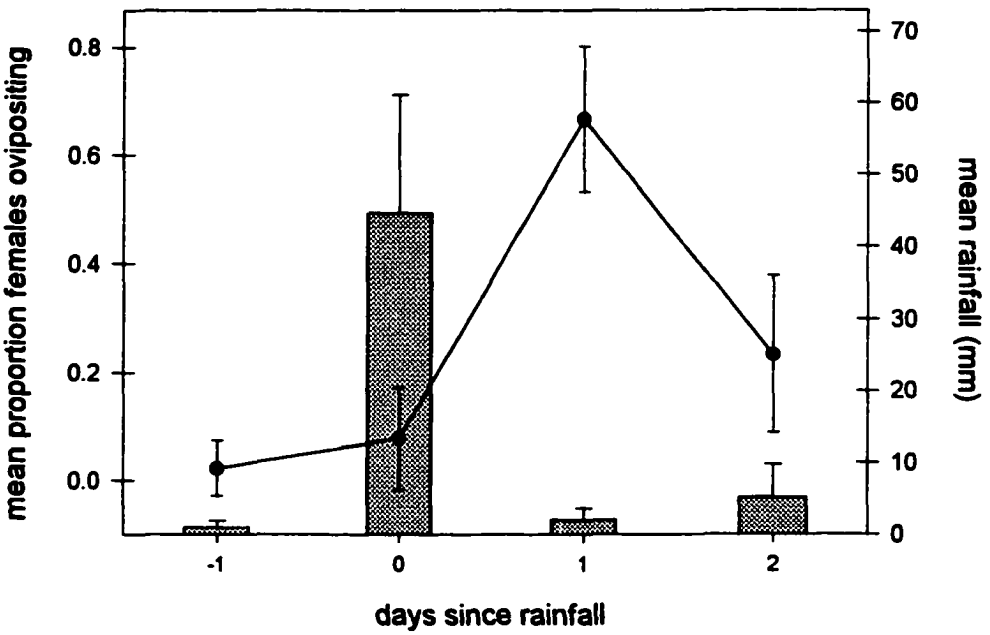

mean proportion females ovipositing
0.8
0.6
0.4
0.2
0.0
70
60
50
40
30
20
10
0
mean rainfall (mm)
-1
0
1
2
days since rainfall

FIGURE 8

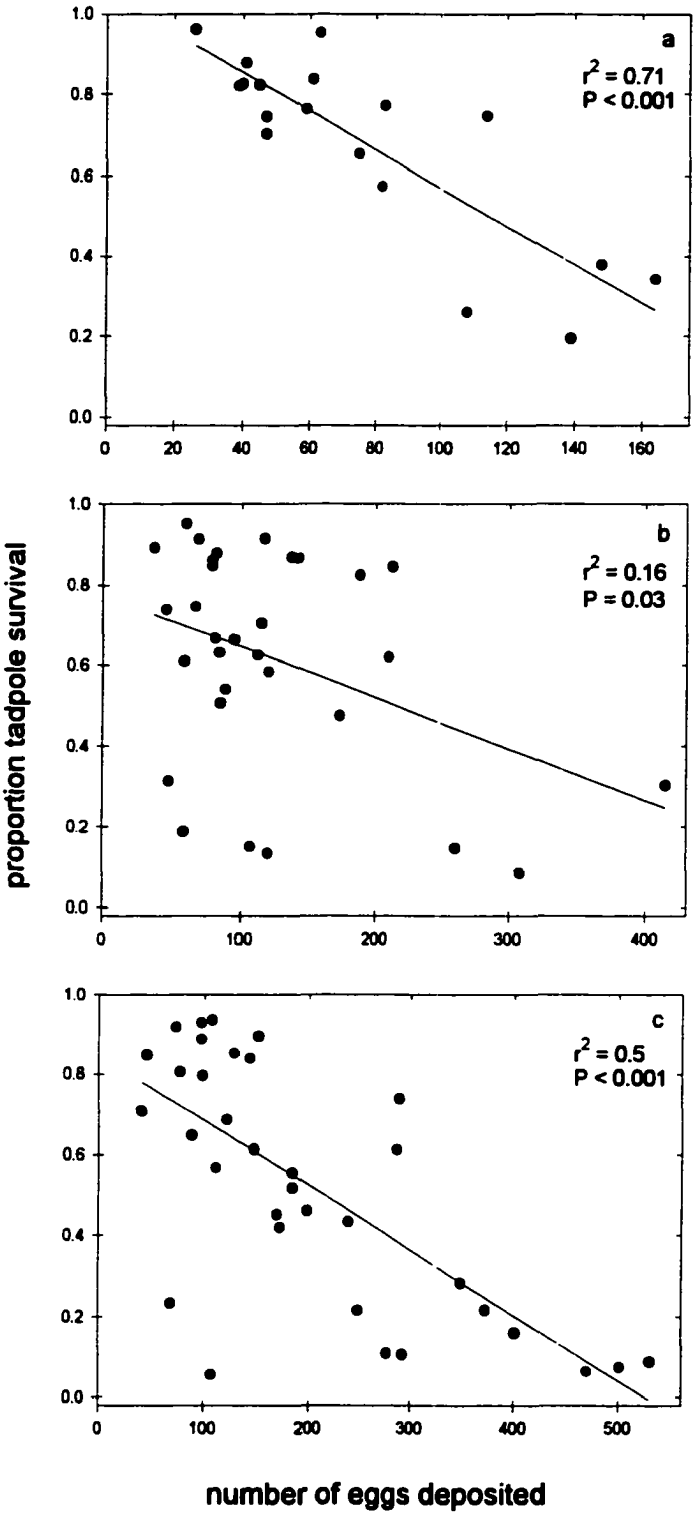

a
$r^2 = 0.71$
P < 0.001
b
$r^2 = 0.16$
P = 0.03
c
$r^2 = 0.5$
P < 0.001
proportion tadpole survival
number of eggs deposited

FIGURE 9

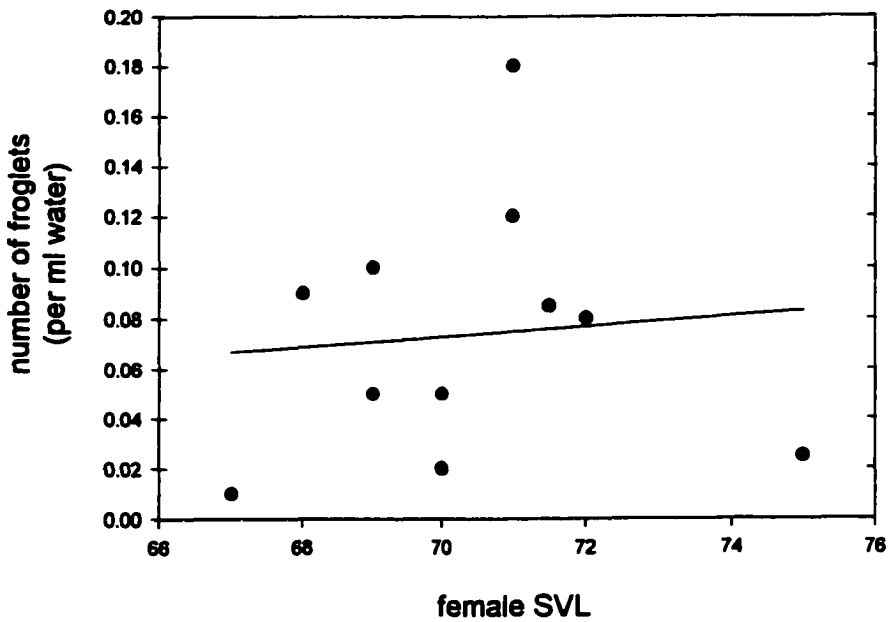

number of froglets
(per ml water)
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
66
68
70
72
74
76
female SVL

FIGURE 10

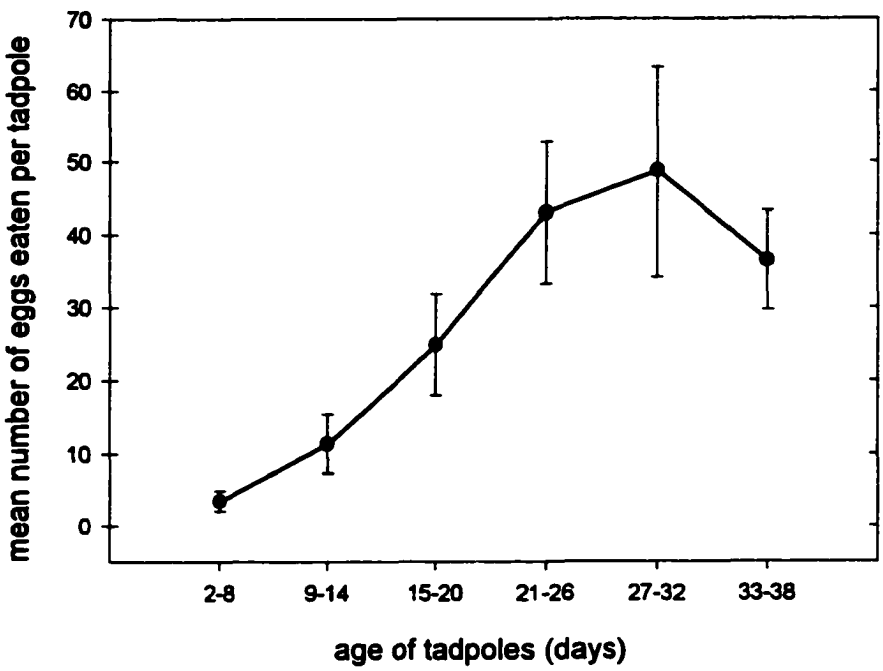

mean number of eggs eaten per tadpole
70
60
50
40
30
20
10
0
2-8
9-14
15-20
21-26
27-32
33-38
age of tadpoles (days)

FIGURE 11

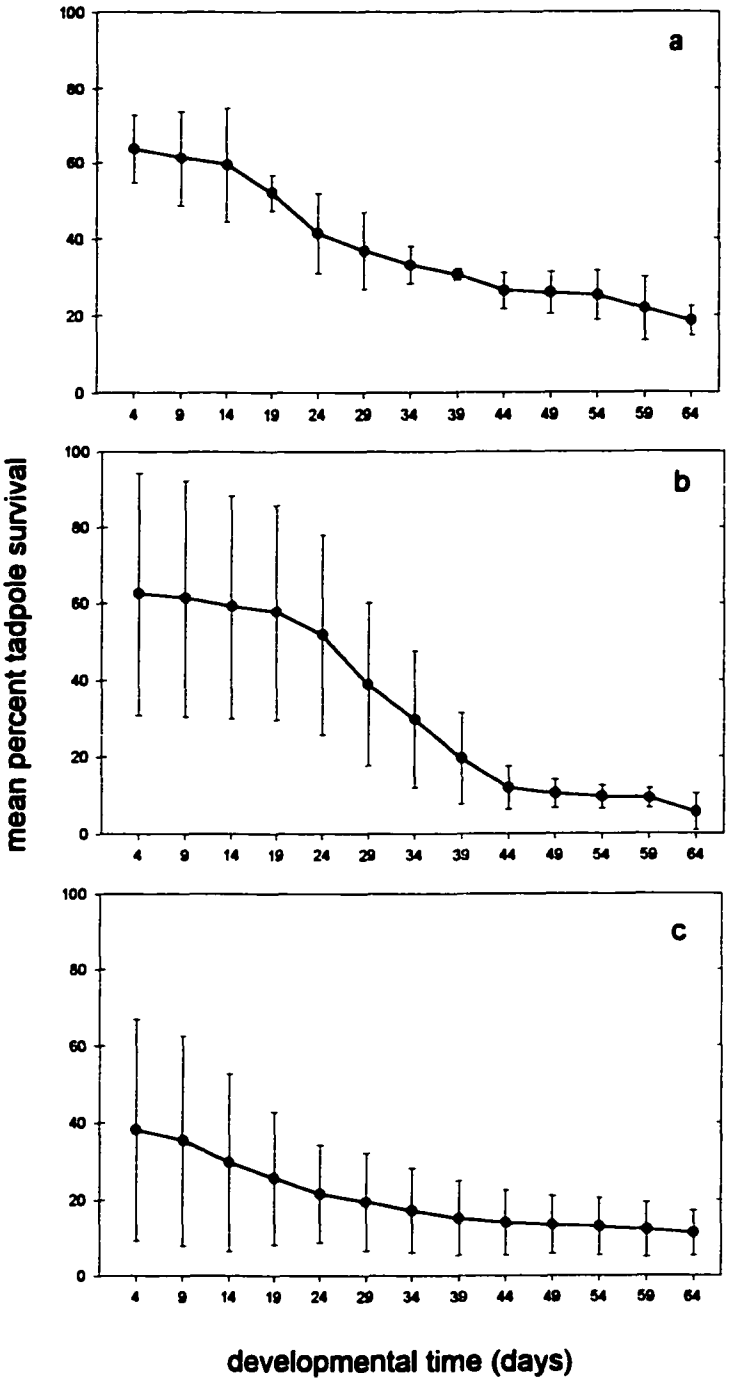

a
b
c
100
80
60
40
20
0
4
9
14
19
24
29
34
39
44
49
54
59
64
mean percent tadpole survival
developmental time (days)

FIGURE 12

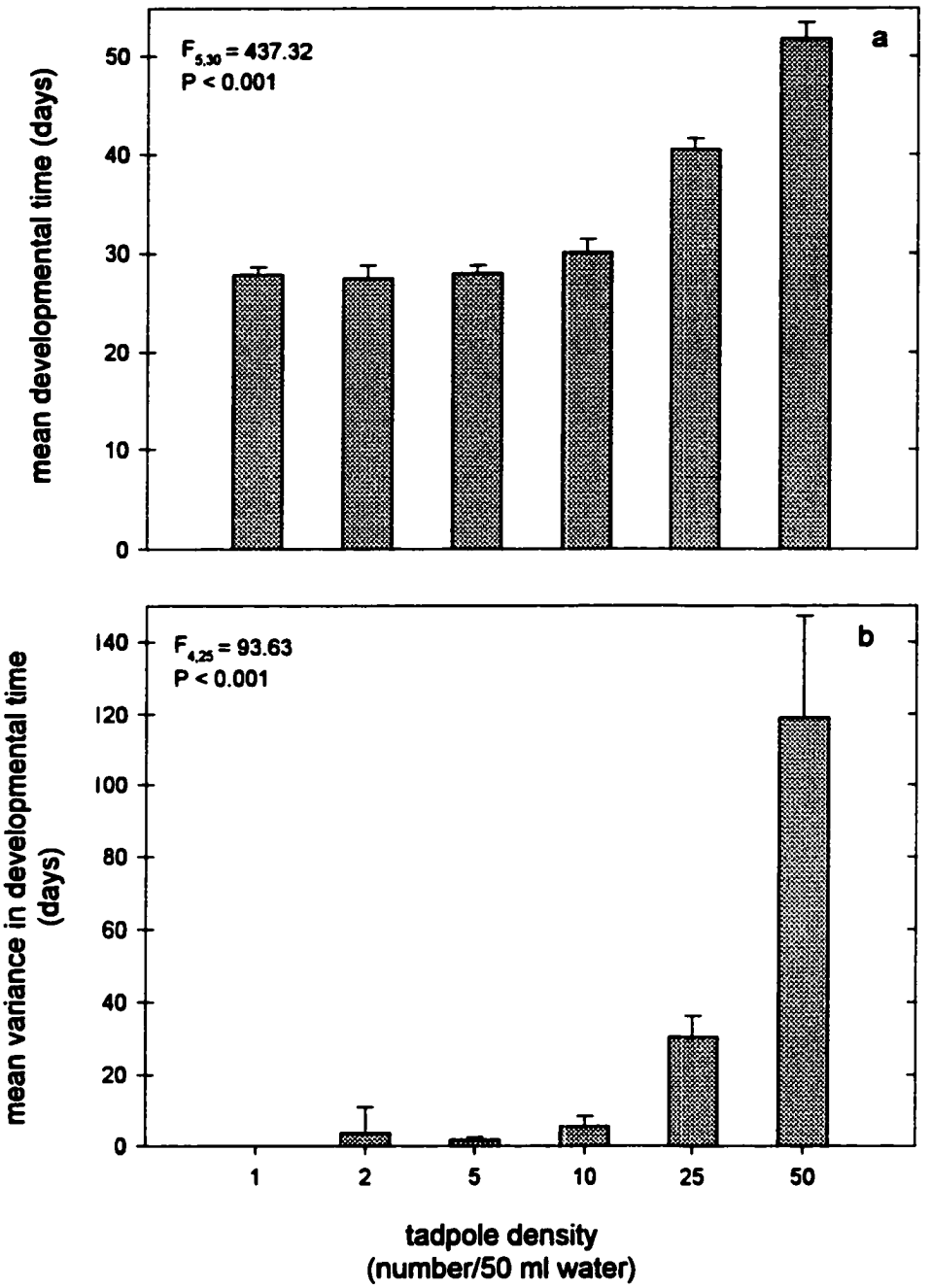

a
$F_{5,30}$ = 437.32
P < 0.001
mean developmental time (days)
50
40
30
20
10
0
b
$F_{4,25}$ = 93.63
P < 0.001
mean variance in developmental time (days)
140
120
100
80
60
40
20
0
1
2
5
10
25
50
tadpole density
(number/50 ml water)

FIGURE 13

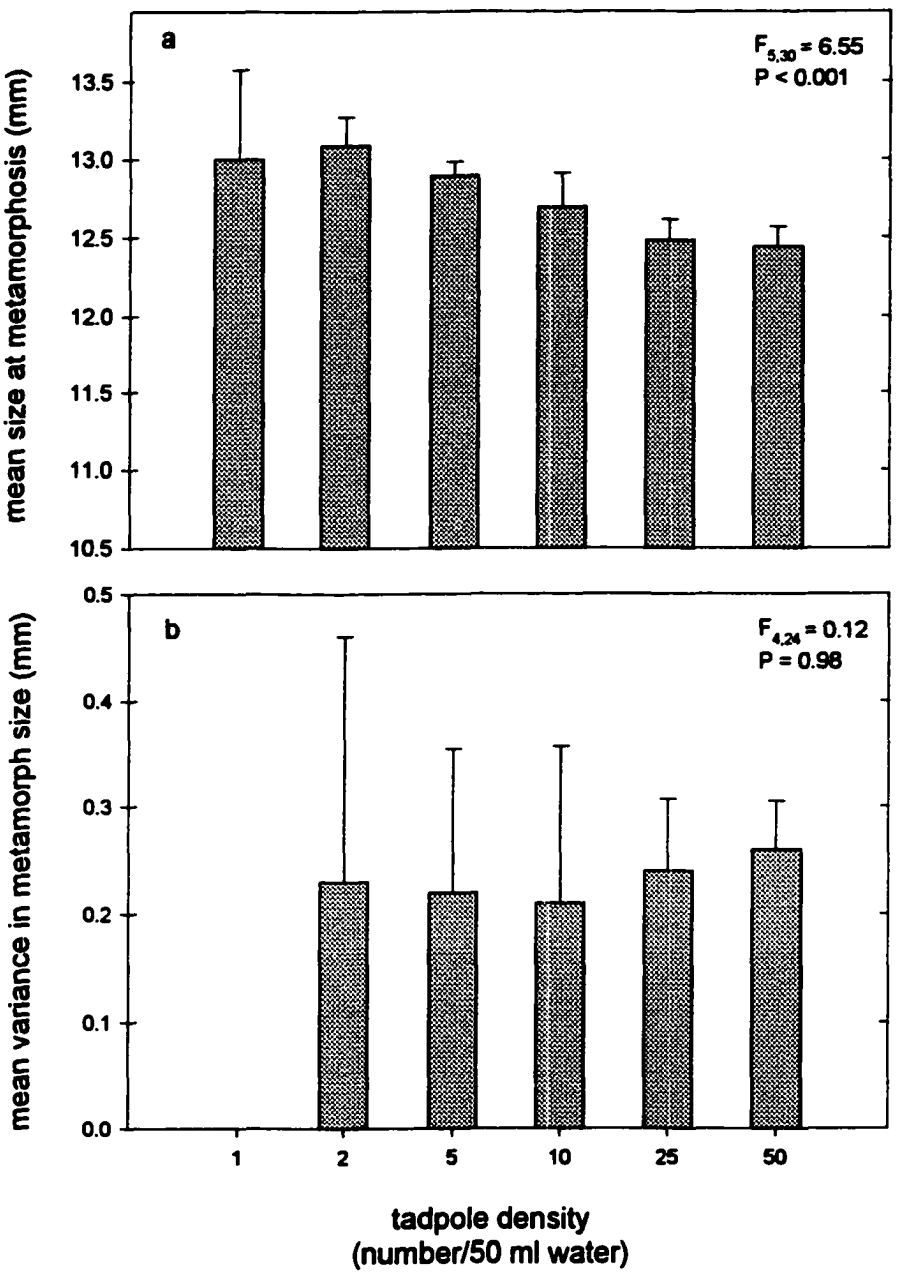

a
$F_{5,30} = 6.55$
$P < 0.001$
mean size at metamorphosis (mm)
13.5
13.0
12.5
12.0
11.5
11.0
10.5
b
$F_{4,24} = 0.12$
$P = 0.98$
mean variance in metamorph size (mm)
0.5
0.4
0.3
0.2
0.1
0.0
1
2
5
10
25
50
tadpole density
(number/50 ml water)

FIGURE 14

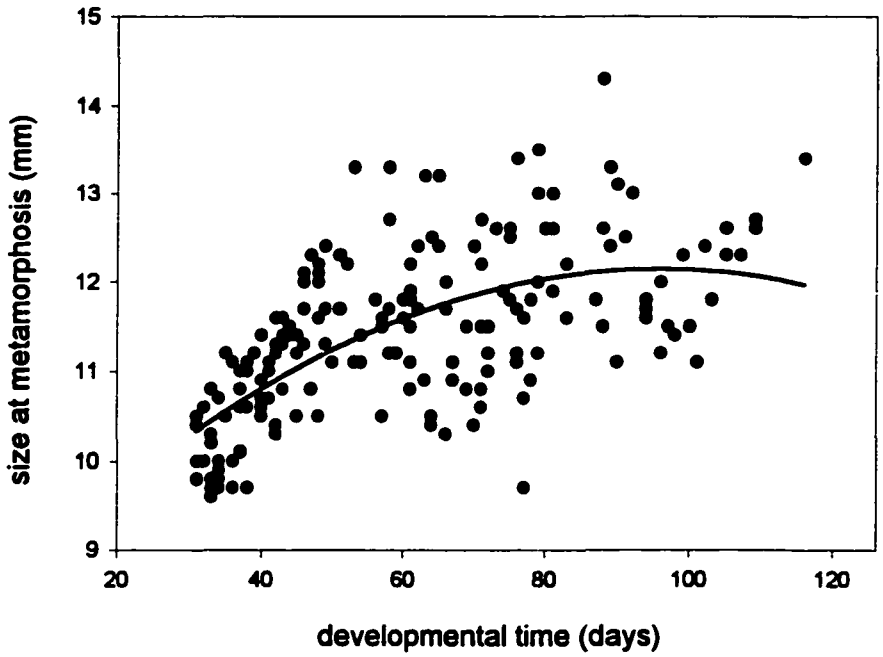

size at metamorphosis (mm)
15
14
13
12
11
10
9
20
40
60
80
100
120
developmental time (days)

FIGURE 15

FACULTATIVE MONOGAMY AND BIPARENTAL CARE IN THE BROMELIAD-BREEDING LAUGHING FROG, *OSTEOCEPHALUS PLANICEPS*

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ABSTRACT

To determine how ecological factors of both offspring and adults can influence the adult mating system, I studied the parental care and mating behavior of the laughing frog, *Osteocephalus planiceps*. This species uses the water-filled leaf axils of bromeliads as larval nurseries. Both male and female laughing frogs are site-faithful, returning again and again throughout tadpole development to remate at the same sites, thereby providing their young with needed nutrients in the form of eggs. Whereas males tend to remain at the oviposition sites continuously and defend them against conspecific males, females leave the sites immediately after mating and do not return until their next egg deposition (roughly a week later). Due to the uneven spatial distribution of the oviposition sites, some individuals mate at clumped sites, while others use more isolated sites. Although there were no differences in mating success for both sexes at clumped vs. isolated sites, the diversity of breeding partners was greater at clumped sites, which featured polygynandrous mating systems, while at isolated sites, frogs tended to be monogamous. At the isolated sites, misdirected paternal care was uncommon, resulting primarily from females physically recruiting new partners to assist in oviposition for existing broods. Such borrowed males thus contributed overwhelmingly to unrelated tadpoles, but the great majority (81%) of trophic eggs were consumed by both parents' own offspring. Male laughing frogs appear to be constrained in their ability to monopolize females by three factors: (i) the limited space in nurseries, (ii) the physiological needs of embryos and tadpoles, and (iii) the tendency of females to breed synchronously in response to rainfall. These generally result in a facultatively monogamous mating system.

Key words: anuran, facultative monogamy, mating system, parental care, reproduction

INTRODUCTION

Animal mating systems are the products of interrelated factors, including, but not limited to, mode of reproduction (i.e., external vs. internal fertilization), amount of parental investment required by offspring, and ecological conditions (i.e., resource distribution) encountered (Trivers 1972; Emlen and Oring 1977). Although discrete categories of mating systems have been defined (e.g., monogamy, polygyny), the mating patterns exhibited by many species are often to variable to pigeonhole, not only between species, but even within a single population, as individuals of both sexes compete to maximize fitness (Davies 1991). Because males can potentially sire offspring at a faster rate than females can produce them, male reproductive success for many species may be limited primarily by access to females, whereas female reproductive success is often limited not by the number of mating partners, but by the availability of resources (Bateman 1948; Trivers 1972; Davies 1991). The difference in these limiting factors suggests that resource availability (e.g., oviposition sites) will determine female distribution, which in turn, are likely to affect male distribution (Bradbury and Vehrencamp 1977; Emlen and Oring 1977). These spacing patterns may become more complex for species in which both males and females provide parental care (Davies 1991).

In amphibians, the primitive life history strategy presumably involved external fertilization of eggs in aquatic habitats, where free-swimming larvae developed through metamorphosis with no postzygotic parental care (Duellman and Trueb 1986). Although most anurans have retained external fertilization, there has been an impressive and variable diversification of reproductive strategies, plausibly due to high predation pressures in aquatic environments (McDiarmid 1978). Within ephemeral catchments of rainwater, many invertebrate predators (such as odonate naiads:

Brockelman 1969) feed on tadpoles, while in temporally more stable habitats (such as ponds), predatory fish may eliminate tadpoles entirely (Heyer et al. 1975). In the tropics there has been an evolutionary trend towards terrestrial breeding among anurans with a concomitant increase in complexity of reproductive strategies (Duellman and Trueb 1986). This may be due in part to the predation pressures encountered by aquatic larvae (McDiarmid 1978), but also to the availability of exploitable microhabitats in the moist humid conditions of such forests.

The evolution of parental care among anurans has presumably allowed many species to gain access to relatively harsh terrestrial microhabitats, where parents compensate by deterring potential predators (Salthe and Duellman 1973), preventing desiccation of anamniotic eggs (Crump 1995), and providing nutrition (in the form of 'trophic eggs') for larvae developing in nutrient-poor microhabitats (McDiarmid 1978; Wells 1981). One class of inhospitable microhabitats invaded as anuran nurseries is the small rainwater catchments ('phytotelmes') of plants, such as the pools in bromeliad leaf axils (Laessle 1961; Lannoo et al. 1987). Though these microhabitats can be relatively stable in terms of longevity (Krügel 1993), they are often food-limited with little or no primary productivity of their own (Laessle 1961). Various morphological and ecological adaptations to the food-limited nature of the microhabitats inhabited by resident tadpoles have evolved (reviewed in Lannoo et al. 1987). While tadpoles of some species are able to develop successfully through metamorphosis by feeding exclusively on the micro- and/or macro-fauna/flora found within the oviposition sites (Lannoo et al. 1987), others rely on nutrition supplied by the mother, either in the form of extra yolk reserves (e.g., *Platyhyla grandis*, *Plethodontohyla notosticta*, and *Anodonthyla boulengeri*; Blommers-Schlösser 1975; *Bufo periglenes*, Crump 1989) or the deposition of unfertilized trophic eggs (e.g., *Dendrobates*

pumilio, Weygoldt 1980, Brust 1993; *Osteopilus brunneus*, Thompson 1992; *Antotheca spinosa*, Jungfer 1996; *Chirixalus eiffingeri*, Kam et al. 1997). The addition of trophic eggs appears to occur only in species for which the tadpoles develop in otherwise isolated pools , such as phytotelmes (Crump 1992), and is probably an adaptation to the limited organic matter contained within those sites (Duellman and Trueb 1986; Lannoo et al. 1987; Jungfer 1996).

Supplying nutritive eggs for tadpoles typically requires only the female parent, but some little-studied *Dendrobates* and *Osteocephalus* male-female pairs are involved in the provisioning process. The male *D. vanzolinii* seems to lead his mate to the sites where isolated tadpoles have been relocated (Caldwell 1997; Caldwell and de Oliveira 1999), while in *O. oophagus* (Jungfer and Weygoldt 1999) and *O. planiceps* (this study) the 'trophic eggs' are themselves freshly fertilized upon deposition, making them genetically complete for development as independent offspring if not consumed. Such involvement of the male partner differs from many other forms of biparental care reported for other taxa (e.g., most birds: Lack 1966) in several key respects. Not only are fertilized 'trophic eggs' complete as proto-offspring, but their creation is the fruit of two highly specialized roles; that is one parent cannot substitute for the absence of the other by simply increasing its own contribution. Rather, if one partner is absent for any reason, the remaining parent has only two options: 1) it can abandon the offspring (in which case, if care is obligatory, the young will perish), or 2) it can try to recruit a new mate to the oviposition site, which, if successful, would result in the recruited individual contributing to the care of at least one cohort of unrelated offspring. This second pattern has not been documented for anurans, though several species of salamanders that attend eggs in communal nest sites exhibit 'alloparenting' behavior where offspring unrelated to the attending par-

ent receive care (Crump 1995).

I investigated the reproductive activities of the laughing frog, *O. planiceps*, a species that seemed likely to shed light on how larval needs and the patchy distribution of nursery sites might shape the parental mating system. The laughing frog, which occurs in the Amazon Basin of Peru, makes use of small catchments of rainwater retained in the leaf axils of bromeliads to deposit its eggs and raise its tadpoles. Each female deposits hundreds of eggs, typically dividing her clutch among several leaf axils of a single bromeliad, and then returns periodically (ca every 10 days) to the same oviposition site, mates, and deposits secondary clutches that most commonly function to provide nutrition for her developing tadpoles. Parental care is obligatory: larvae not provided with nutritive eggs do not survive through metamorphosis (Haugen, Chapter 1).

In species where limited resources necessary for reproduction vary in quality or are unevenly distributed a potential exists for obtaining multiple mates (Emlen and Oring 1977). A limiting resource for many anurans, including laughing frogs, and one that is often defended by males against conspecific rivals, is the oviposition site. Wells (1981) cites several anuran species in which territorial males, attending one or more clutches, continue to call and attract additional females. For some (e.g., *Rana catesbeiana*, Howard 1978a, b), oviposition sites are known to vary in quality with the males defending better quality sites experiencing greater reproductive success than males with less optimal sites. For other species, the determining environmental factor affecting individual reproductive success may be the spatial distribution of oviposition sites. Single males may obtain a disproportionate amount of matings by defending sites that are unevenly distributed and spatially clumped (Emlen and Oring 1977).

The mating system of the laughing frog may have evolved in response both to ecological conditions (e.g., distribution of oviposition sites) and phylogenetic constraints (e.g., the need for iterated parental care). The oviposition sites used by laughing frogs have a naturally uneven spatial distribution, such that some bromeliads occur in clumps while others are more isolated. To determine if the relative proximity and thereby defendability of oviposition sites affects the mating system of the laughing frog, I manipulated the distribution of available sites in a study area within which a discrete population of laughing frogs occurred. If some males are able to defend multiple oviposition sites where clumped and monopolize females, then those males should have a greater variance in mating success than do males at isolated sites. To determine if parental care affects the mating system, I examined the extent of parental care in both male and female laughing frogs and the effect that male desertion, through both experimental and non-experimental removals, has on female parental behavior. If paternal care is obligatory, the ability of males to monopolize females may depend not only on the spatial distribution of the oviposition sites, but also on the temporal distribution of females.

METHODS

Study site

Laughing frogs are prolonged breeders and individuals mate throughout the year. They are found in disturbed habitats (e.g., forest gaps) where they oviposit in the bromeliads still attached to fallen trees. I studied a discrete population of laughing frogs at the Amazon Conservatory of Tropical Studies (formerly the Amazon Center for Environmental Education and Research), located in the upper Amazon Basin in

Departamento Loreto, Peru (3°58'S, 72°59'W), Jan. 1996 to June 1996. Surrounded by primary rainforest, the 3600 m^2 study area consisted of both cleared habitat (near the station buildings) and secondary growth. The nearest breeding population in unmodified habitat was 350 m away in a large natural gap.

I investigated the determinants of this species mating system with a mix of natural oviposition sites (i.e., bromeliads) and artificial oviposition sites. Prior to my study, bromeliads collected from nearby tree falls were tied to trees in the area around the station. To increase the number of oviposition sites and to facilitate monitoring of eggs and tadpoles, I attached artificial catchments (plastic cup assemblages) to trees. My preliminary observations indicated that females divide their clutches among 3 - 5 bromeliad axils when depositing eggs. Each artificial oviposition site, therefore, consisted of five 250-ml clear plastic cups with pin-hole punctures in the sides to maintain various amounts of water that represented the range naturally found within bromeliad axils (25 – 100 ml). I maintained water volumes by adding extra water (daily, if needed). I placed each cup separately within an opaque 700-ml green plastic cup that I had wired to a tree. I positioned the cups at various angles to approximate the depth and surface area of the pools of water held within bromeliad leaf axils. By utilizing holders for the smaller transparent cups, I could remove the inner cups and readily count number of eggs deposited and monitor tadpole survival, while maintaining the exact location and position of each cup. The green holder cups were perforated at the bottom, thus did not retain rainfall.

To determine the number of oviposition sites utilized and the possible range of areas successfully defended by males, I relocated certain bromeliads and positioned some artificial catchments so that most oviposition sites were <u>isolated</u> (at least 5 m from nearest neighbor) while others were <u>clumped</u> in space (i.e., 2 - 5 natural and/or

artificial sites located within 2 m of one another). In all, about 50 bromeliads and 15 artificial sites (of 5 cups each) were available in the study area. All oviposition sites in the area (both bromeliads and cups) were located 0 - 3 m above ground.

Field data collection

Each night from 1800 to 0200 h I censused the study area, checking all oviposition sites at least twice per hour and recording the location of each individual frog and the time sighted.

Upon an individual's initial capture, I assigned it a number and recorded sex, snout-vent-length, and mass, along with location and time of discovery. To identify individuals on subsequent sightings, I made sketches of their distinctive dorsal markings. To assure that no two individuals had the same markings, I clipped the inner toe pad (digit I from one hind limb) as a secondary identification to indicate that the individual had been previously captured and recorded. Because I limited the clipping to the fleshy toe pads, I re-clipped them periodically as they tended to grow back.

When a new female arrived in the study area, I either remained in the vicinity of the oviposition site or I increased my visit frequency to her location so that I could determine the identity of the amplexed male and the site and time of oviposition. The serial deposition of a clutch (typically divided among several leaf axils) by an amplexed male-female pair will be referred to as a mating event and mating success will be defined by the number of mating events per individual. Females tended to arrive before 0200 h and rarely mated after 0200 h. If a focal female had not deposited eggs by 0200 h, I checked her every half hour throughout the night until she oviposited. For two of the females that mated at clumped sites, I was unable to determine the identity of their mates due to the location of the bromeliad and the num-

ber of males present at the time of oviposition. Because I was unable to determine with certainty the number of partners these two females mated with, I excluded them from an analysis comparing the number of different males females mated with at clumped and isolated sites.

I recorded matings at both artificial oviposition sites and bromeliads. When eggs were deposited in cups, I recorded the specific locations of the egg masses and the number of eggs contained in each. Because females typically distribute their clutches among several cups, I refer to eggs deposited in separate cups as 'sub-clutches' ('sub-broods' when the eggs had hatched to produce tadpoles). To distinguish between clutches deposited in sites devoid of tadpoles and clutches deposited repeatedly in the same sites by either a male or female parent, I refer to the first clutch deposited as the initial clutch (or an initial sub-clutch); clutches deposited thereafter, secondary clutches (or secondary sub-clutches).

Tadpoles began to hatch ca 36 h after egg deposition and by 3 - 4 d post-oviposition all surviving tadpoles in a brood had hatched and were free-swimming. I generally did not disturb the developing embryos until 4 d post-oviposition. From that point on, I kept daily records of tadpole survival through metamorphosis in the artificial sites.

I observed male-male conflicts at both artificial oviposition sites and bromeliads opportunistically throughout each night as I censused the study area. When I saw males fighting (i.e., jumping on one another or wrestling for access to an oviposition site or female), I recorded the location of the site (i.e., clumped or isolated), identity of the males and, if a female was present, the identity of the males(s) that eventually mated with the female.

Relatedness

To ascertain correctly whether care throughout larval development was directed towards related or non-related offspring, I include in analyses only those clutches for which I had positively identified both members of the original ovipositing pair. This precaution limited analyses to clutches deposited in isolated sites due to the probability of multiple paternity at clumped sites. Because the eggs are externally fertilized, I could assign parentage with reasonable assurance at the isolated sites where single male-female pairs mated.

Male removals

To determine if females with young abandon oviposition sites or deposit unfertilized eggs when males are consistently absent from the sites, I removed males from eight oviposition sites in May 1996. I included every available site in the study area in which tadpoles were less than two weeks old and for which I had identified the depositing female. Because two of these sites were each visited by two different females, the removals affected a total of 10 females. At each targeted site throughout the month, I removed the resident male two days before each anticipated return of his mate. Removed males were housed in the research station. When females arrived to deposit eggs, I recorded the length of time each waited (presumably for their mates to return). If the female left the site to recruit a new male, I recorded the distance she traveled and the identity of the borrowed male. To discourage newly recruited 'replacement' males from establishing territories at the experimentally vacated sites, I released each resident male back at his original site after his mate had departed and removed him again prior to his female's next return.

Data Analysis

Throughout, means are reported ± 1 SD and comparative tests are two-tailed. SYSTAT 9 for Windows was used for all analyses (SYSTAT 1998) except those indicated below. To test for equality of group variances, I used Levene's test for unequal variance. The Aspin-Welch-Satterthwaite (AWS) test, which is robust to unequal population variances when sample sizes are small and/or unequal (Toothaker and Miller 1996), was used in place of the two-independent-sample *t*-test. Levene's test statistic, tests for normality, regressions and power analyses were calculated using JMP IN statistical software (SAS 1997). When the test for normality did not indicate a normal distribution, I used the nonparametric Mann-Whitney U test in place of the AWS test. The G-test of independence (which tests the goodness of fit of the observed frequencies to their expected frequencies, BIOMstat 1996) was used to determine if the occurrence of male-male conflicts was independent of the proximity of oviposition sites (i.e., clumped vs. isolated). To lower the probability of type I errors, I adjusted the G-test statistics using Williams's correction (Sokal and Rohlf 1995).

RESULTS

Between 24 January and 4 May 1996, I observed 242 mating events between a total of 31 females and 36 males. The matings occurred in 26 natural oviposition sites (135 clutches deposited repeatedly in 14 bromeliads by 18 females and 24 males: mean = 11.3 ± 6.9 depositions per site) and 12 artificial cup sites (107 clutches by 14 females and 17 males; mean = 9.3 ± 5.4). Most individuals mated exclusively at either bromeliads or artificial sites, but one female and five males used both types. Although females obtained a greater number of mating events per individual (6.7 ±

4.1, $n=36$ females) than did males (4.3 ± 4.7, $n=51$ males; AWS $t_{80.7}=2.52$, $P=0.01$), there was no demonstrable difference in the variance of mating success of males and females (Levene's test for unequal variances: $F_{1,85}=0.70$, $P=0.41$).

Typically, males established territories at the oviposition sites and attracted females to those sites by calling. I detected females, both when they entered the study area from the surrounding forest prior to mating and as they returned to the forest after depositing eggs. Males tended to stay at their oviposition sites. Once mated, females returned reliably to the same sites repeatedly for re-mating. Females at isolated sites that had been deserted by the original male (or that had located a suitable and unoccupied oviposition site) attempted to recruit new males to these sites. In this process, she allowed a nearby male to amplex, and then carried him to her site.

Of the 242 total mating events observed, 106 occurred at three clumped sites (each consisting of 3-6 bromeliad and artificial sites) and 136 occurred at 14 isolated sites. Mean nearest neighbor distance, including all utilized sites, was 9.5 ± 3.1 m. The three clumped sites were situated 24.9 ± 1.6 m apart (see Appendix I for site map).

Adults known to have mated tended to remain longer (span from first to last observation) than those not mating (AWS $t_{57.4}=12.55$, $P<0.001$) and roughly half of the known individuals of both sexes (15 males, 16 females) remained in the area throughout the entire 102 d study. Non-mating males visited the area only briefly (15 such males averaged 12.7 ± 18.4 days, while the 5 non-mating females stayed only 3.4 ± 4.3 days) and half of these (7 males, 3 females) were seen only once or twice, thus presumed to be transients.

To examine patterns of philopatry, I trimmed the total sample of mating events

to remove adults observed reproducing only once. Of the individuals that I observed mating more than once (7.2 ± 4.2 mating events per male, 7.8 ± 3.3 mating events per female), the 30 males mated at more sites (2.0 ± 1.2 sites) than the 31 comparable females (1.1 ± 0.3 sites; AWS $t_{33.3}=3.74$, $P=0.001$). Indeed, nearly all such females (27/31) were seen to mate at only one site repeatedly throughout the study. Of the four exceptions, one female switched to a new site after her original site was destroyed, and another abandoned her original site to predators (carnivorous *Dendrobates ventrimaculatus* tadpoles that readily consume laughing frog tadpoles) after she had failed in trying to recruit a male to the site. The other two females deposited eggs in the oviposition sites of males they were attempting to recruit back to their own broods.

Male-male conflicts

Males actively defended both their mating partners and their territories' oviposition sites (bromeliads and/or cups) from conspecific males through physical combat. I documented 50 fights between males, mostly (68%) with a female present. The majority of conflicts (76%) took place at bromeliads and cups located within clumped sites. Considering that 50% of all oviposition sites were located within clumped areas, fights appeared to be nonrandomly associated with such locations ($G_{adj}=14.07$, df = 1, $P<0.001$). Eight of the fights resulted in a male abandoning the site.

On 34 occasions I observed a female arriving at an oviposition site with multiple males already present: up to six males competed for sole access to her by jostling one another and attempting to amplex. The female typically remained passive while the males tried either to hold on tightly or to pull others off. Females usually did not begin ovipositing until only one male remained in amplexus. Only one of these multi-

ple-partner mating events occurred at an isolated site.

Matings at clumped vs. isolated sites

To assess how the spatial distribution of oviposition sites may have shaped the basic mating system of laughing frogs, I focused initially on the most successful mating adults (operationally defined here as those participating in three or more mating events with their primary partners). This approach temporarily excluded extra-pair fertilizations (resulting from females that borrowed neighboring males). Of these multiple-mating adults, roughly half (15 females and 15 males) used bromeliads or cups at isolated sites, and half (13 females and 11 males) used those at clumped sites. There was no difference in the number of matings obtained by individual females at isolated (7.9 ± 2.8, range 4 to 13, $n=15$) and clumped sites (7.7 ± 2.7, range 3 to 12, $n=13$; AWS $t_{24.7}=0.23$, $P=0.82$). Likewise, individual males at isolated sites mated as often (7.4 ± 4.4, range 3 to 19, $n=15$) as those at clumped sites (6.9 ± 3.7, range 3 to 15, $n=11$; AWS $t_{23.4}=0.31$, $P=0.76$). Further, the variance in male mating success (operationally defined as number of mating events per individual in which amplexed male-female pair deposited eggs) was similar at clumped (CV = 58.9%) and isolated sites (CV = 53.6%) (Levene's test for equality of variance: $F_{1,24}=0.41$, $P=0.53$); as it was for females (CV = 35.5% vs. 35.4%; $F_{1,26}=0.21$, $P=0.65$). For both males and females, mating success was related to the total time an individual that had remained at a given oviposition site (males: $r^2_{adj}=0.24$, $F_{1,25}=9.03$, $P=0.006$, Fig. 1; females: $r^2_{adj}=0.61$, $F_{1,26}=42.78$, $P<0.001$, Fig. 2).

Even though the number of mating events per individual did not differ for clumped and isolated sites, individuals at clumped sites tended to have greater num-

bers of mating partners than did those at isolated sites. While none of the individuals at clumped sites mated with just one partner, 9 pairs formed at the isolated sites and remained sexually exclusive, mating an average of 5.3 ± 2.6 times (range 3 to 11 matings per pair). The eleven males at the clumped sites mated with more females (median 3) than the 15 males at isolated sites (median 1; Mann-Whitney $U = 156$, $P < 0.001$, Fig. 3). Eleven females at clumped sites similarly mated with more males (median 4) than did the 15 females at isolated sites (median 1; Mann-Whitney $U = 153.5$, $P < 0.001$, Fig. 4).

Although females at clumped sites tended to mate with more partners than females at isolated sites, they were just as site-faithful to a particular bromeliad (natural or artificial) as females at isolated sites. Each female mating at a clumped site, deposited her clutches in the same bromeliad or set of cups each time she mated. The males at clumped sites showed no such specific site fidelity, but instead mated at whichever catchments the amplexed female chose. Thus, males at the clumped sites used more bromeliads and cup set-ups for oviposition (median 3) than did males at isolated sites, where each male used just the one bromeliad or cup set-up per site (Mann-Whitney $U = 157.5$, $n_1 = 11$, $n_2 = 15$, $P < 0.001$).

Parental care at isolated sites – how much of it is 'misdirected'?

Following oviposition at sites in which the parents had previously deposited sub-clutches, two mutually-incompatible fates generally await the zygotes, either of which can enhance parental fitness. If the nursery is unoccupied (as occurred for 8.3% of the sub-clutches placed in isolated cups), many of the embryos can survive to metamorphose as froglets. But if the nursery is already occupied (91.7% of sub-clutches), the newly added eggs invariably served as food for the incumbents. When

the tadpole residents are siblings of the doomed eggs, the victims gain indirect fitness through their nutritional contribution to kin and the parental act of producing them can be viewed as parental investment (Trivers 1972). The amplexing pair's new investment is 'misdirected' (*sensu* Alexander 1974) when the incumbent consumers are unrelated to either or both parent. Overall, the 14 individually recognizable females and 12 males were highly (and equally: AWS $t_{20.3}=0.04$, $P=0.97$) successful in getting new eggs (sub-clutches) to their own tadpoles (Table 1).

Lost fitness from misdirected parental investment at isolated sites might accrue occasionally to females from various cognitive errors (e.g., locating and using repeatedly an oviposition site containing another female's offspring: 2 females, 13 cases observed) or special circumstances (e.g., depositing eggs in catchments of neighboring male prior to recruitment attempt: 2 females, 7 cases observed). Males were most likely to misdirect parental investment because of being actively recruited as replacement partners. The vast majority of such male recruitment events observed (Table 2) allowed the feeding to tadpoles related to the participating female, but not to the recruited male. On the other hand, 8 of the 66 sub-clutches (12.1%) produced by females accompanied by such replacement males (including 3 sub-clutches deposited in the males' sites prior to recruitment) were placed in nurseries not containing older resident larvae. These presumably carry the same potential for reproductive success as any primary sub-clutch.

Prior to the male removal experiments, adults of both sexes exhibited misdirected parental investment by depositing fertilized eggs into catchments containing tadpoles unrelated to them. Including all eggs deposited (i.e., initial plus secondary sub-clutches), males appeared to average nearly two times as many misdirected egg depositions (28.8% ± 34.4% of all sub-clutches, $n=18$ males) as did females

(15.0% ± 23.1%, $n = 14$ females), though this difference was not statistically significant given the high variance and small sample size (AWS $t_{29.5} = -1.35$, $P = 0.19$; power = 0.20). A sizeable proportion of adults (43%, $n = 6$ females; 39%, $n = 7$ males) never deposited into catchments containing unrelated tadpoles. Although not quantified, misdirected parental investment presumably occurred at a higher frequency in the clumped oviposition sites where more individuals deposited clutches in the same catchments.

Male absences

At the clumped sites, males were always present when females returned to deposit secondary clutches, while at the isolated sites, males were absent during 23 (17%) of the 136 mating events. Eleven of the 23 male-absences resulted from snake predation of the resident males; in seven others, the male was delayed for unknown reasons, but still managed to reach the site in time to rendezvous with the female and mate. Thus, on only five occasions (4% of 125) were living males not present in time to mate with the returning female. Of these exceptions, one male was the congeneric species, *O. taurinus* that had deposited a clutch with an *O. planiceps* female. The 23 male absences affected a total of 14 females; no female deserted her brood due to the absence of the male, but instead returned repeatedly to oviposit.

Upon failing to encounter a male at her site, a female either left immediately, returning the next night to try again, or she waited nearby. If the male did not appear, the female attempted to recruit a new male to her site. At no time did I observe a single female deposit unfertilized eggs in aquatic catchments, with or without tadpoles. The waiting periods for the four females that did not leave their sites, but whose partners eventually showed up (mean = 2.6 ± 3.0 h) closely matched the time

waited by six females that left their sites to recruit new males (mean = 2.3 ± 0.8 h; AWS $t_{3.3} = 0.23$, $P = 0.83$).

Each of the six recruiting females went to the site of another male, allowed him to amplex, then attempted to carry him back to her own site. Males were absent from the oviposition sites of three of these females on more than one occasion and these females repeatedly retrieved the same substitute males. Two of these females deposited partial clutches (one female on three occasions; the other female, four) in the males' sites before taking them to their own (of the 25 sub-clutches deposited in the males' sites by these two females over time, nine were deposited in sites devoid of tadpoles and became initial sub-clutches, six were consumed by tadpoles unrelated to the females, and ten were consumed by the females' offspring). In all, females attempted to transport males 16 times and were successful in 14 of these in getting them to the females' oviposition sites (one individual male refused to leave his own position on two separate occasions, both times the female deposited a clutch at his site). The mean distance males were transported was 15.1 ± 4.8 m (range 8.4 m to 19.4 m) and once the eggs were deposited, most (12/14) returned immediately to their own oviposition sites.

Experimental removals

The ten additional females whose male partners were removed experimentally (mean 3.0 ± 0.7 female visits, range 2 to 4) continued to return to their original oviposition sites. Four of these females left the study area on one occasion each when the resident males were absent, returning the following night. Females that stayed, waited, on average, 2.6 ± 1.7 h before leaving their sites to recruit new males. The mean distance these males were transported was 15.5 ± 6.9 m (range 6.5 to 27.7 m) with

seven of the 10 females returning to sites from where they had previously retrieved males. I did not observe females abandoning their oviposition sites due to the repeated absences of males.

DISCUSSION

The mating system of the laughing frog, *Osteocephalus planiceps*, appears to center on facultative monogamy (owing mainly to larval food requirements and various biotic and abiotic forces that eliminate whole broods), but which readily manifests itself into more pluralistic forms (especially polygynandry) where nursery sites are spatially clumped. This Neotropical tree frog breeds in the water-filled leaf axils of bromeliads located in disturbed habitats. The egg/tadpole nurseries are intermittently hypoxic and utterly incapable of providing adequate food throughout the 7–12 week period of larval development (Laessle 1961; Haugen, 1). Females tend to oviposit when dissolved oxygen concentrations are relatively high (after rainfall). And by creating a large clutch initially and then depositing additional eggs in the same catchments repeatedly throughout tadpole development, laughing frog parents provide offspring with essential nutrients (Haugen, Chapter 1). Because these nutritive eggs are also fertilized, they simultaneously carry the potential for becoming replacement offspring if the initial brood has died prior to metamorphosis (Haugen, Chapter 1). The special requirement that all egg masses be fertilized (presumably to gain the duality of function) is likely to have imposed an evolutionary constraint on parental options, essentially forcing obligate biparental investment and, in the process, facilitating an iterative social relationship between the two original mates.

Both male and female laughing frogs show great site-tenacity, with the former tending to remain at or near the pair's oviposition site(s) and the latter departing for 7-10 day absences. The returning female is approached by her partner and they oviposit again and again at the same sites. During the 4-month study in 1996, nearly all females (27/31) used one oviposition site exclusively and males averaged just two sites (single sites where spatially isolated; more where clumped).

The basic pattern was disturbed if a returning female did not find her partner on-site. At relatively clumped oviposition sites, alternative mating partners were always available because multiple males tended to occupy those sites. At isolated sites, however, males were absent on 23 of 136 female arrivals and such females often waited for the male to re-appear and then mating proceeded normally (this result was observed in 7 of 12 such occasions when the missing partner was known to remain alive). Nearly 90% of the newly produced egg sub-clutches went into nurseries already containing tadpoles and were eaten promptly; the remaining one-tenth of egg masses went into unoccupied catchments and began development. Of those eaten, over 80% went to feed the elder genetic offspring of the amplexing pair.

When the missing male did not appear (16 such occasions) the female 'borrowed' a mating partner (approached a neighboring male for amplexus and then carried him bodily to her site. The fitness costs accruing to such male are probably quite modest (a bit of time and gamete metabolism that mainly feeds unrelated tadpoles). The compensating benefits for a borrowed male may include some oviposition by the new female at sites containing his own young (observed on seven occasions) plus an estimated 10% chance that new egg masses will end up in unoccupied (i.e., recently depredated) nurseries, where they can develop.

In the types of biparental care found in other taxa (notably birds), the potential exists for one parent to compensate partially for reduced care by its partner (see models by Chase 1980, Houston and Davies 1985). No parallel opportunities exist for laughing frogs because parents provide exogenous nutrients exclusively in the form of fertilized eggs, the one commodity neither parent can provide single-handedly.

The relative costs and benefits of parental care to each sex (and the antecedent condition within the species) are believed to affect the evolution of care and to determine the extent of that care by one or both parents (Maynard Smith 1977, Clutton-Brock 1991). Specifically, biparental care should evolve only when it increases the reproductive success of each parent more than would be expected from the alternative strategy of deserting and seeking additional mates (Maynard Smith 1977, Perrone and Zaret 1979). If a female laughing frog were to desert, her brood would most certainly starve. If a male laughing frog deserts, his absence may not adversely affect his offspring because the female will likely obtain replacement males each time she returns to the site. Males do, however, stay and defend their oviposition sites and continue to mate at those sites. With each mating there is a 10% chance the eggs will be deposited in a catchment devoid of tadpoles – the same probability he would encounter if able to obtain a mating elsewhere. Although males new to the study area tend to roam from site to site, very few use this tactic as a permanent strategy. Instead, males fight for access to oviposition sites even when they have clearly not sired any of the young. Due to the highly unpredictable nature of the sites, a male that is successful in obtaining a site will eventually contribute gametes to viable offspring, though he may invest in unrelated tadpoles in the interim.

Because male laughing frogs defend a resource that is essential to reproduction

and unevenly distributed, they can theoretically increase their reproductive success by monopolizing females (Emlen and Oring 1977). There appears to be an *environmental potential for polygyny* at the clumped sites (due to the multiple females breeding at those sites) that is not realized. Although males defend oviposition sites and fight over access to females, no difference in mating success variance could be demonstrated between males and females. Moreover, the variance in mating success was equal for males at clumped and isolated sites, indicating that individual males at the former do not monopolize females.

Emlen and Oring (1977) recognized that individual males have less opportunity to monopolize females that breed in greater synchrony. They further suggested that the temporal breeding patterns of individuals are influenced by environmental factors. In the laughing frogs habitat, heavy sporadic rains dramatically increase the oxygen concentration in bromeliad water (Laessle 1961, Haugen, Chapter 1), which is critical for anuran embryonic development (Adolph 1979). Female laughing frogs tend to adjust their interclutch intervals in response to heavy rains, producing an overall synchronous pattern of breeding (Haugen, Chapter 1). This temporal crowding means that individual male laughing frogs become less able to maintain exclusive control of more than one oviposition site simultaneously. In short, due to the physiological needs of the offspring, males are constrained in their ability to monopolize females. It appears that the best solution for most males is to participate in extra-site fertilizations opportunistically, while remaining predominantly site-faithful to contribute to the care of their primary broods.

Relatively little comparative literature on similar anuran systems exists to date. One congener, *O. oophagus*, has been reported to exhibit a reproductive strategy similar to that of laughing frogs (amplexing pairs deposit fertilized eggs repeatedly in

phytotelmes: Jungfer and Weygoldt 1999). And one other anuran, *Osteopilus brunneus*, has been observed, under natural conditions, depositing fertilized eggs into catchments that already contained related oophagous tadpoles (Thompson 1992). Tadpoles of a few other species also cannibalize conspecific fertilized eggs (e.g., *Dendrobates ventrimaculatus*, Summers and Amos 1997; Summers 1999; *Mantella laevigata*, Heying 2001), but these may be examples of "reproductive parasitism" on the part of the males. That is, males are known to lead egg-bearing females to nurseries containing tadpoles unrelated to the females (Summers et al. 1999), presumably benefiting the male's fitness but not that of the females. However, these species all differ from the two *Osteocephalus* in that the females have evolved the ability to deposit unfertilized trophic eggs and can effectively provide their offspring with nutrition in the absence of the male.

The *Osteocephalus* pattern, combining biparental care with the deposition of fertilized nutritive eggs, may represent an early step in the evolution of uniparental female care (specifically, the deposition of unfertilized trophic eggs), as suggested by Jungfer and Weygoldt (1999), or it may be specially adapted to the unpredictable nature of small, food-limited oviposition sites. There are at least two selection pressures on both male and female laughing frogs to maintain site fidelity, both of which concern larval ecology: (i) the need for fresh pulses of exogenous food and (ii) frequent annihilation of developing broods (by asphyxiation, predation, etc.). In laughing frogs, the key phylogenetic determinant of the monogamous mating seems to be larval ecology and how it interacts with the spatial dispersion of nurseries and the temporal patterns of rainfall. Comparative work needs to done across taxa to illuminate the interactions among parental options, offspring needs, and the resultant mating systems. Species that exhibit obligate biparental care (i.e., fertilized trophic

eggs), rely on unevenly distributed (spatially and temporally) resources for reproduction, and whose offspring encounter periodic biotic and abiotic disasters will be expected to also exhibit variable mating systems that center on facultative monogamy.

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Appendix A. Site Map

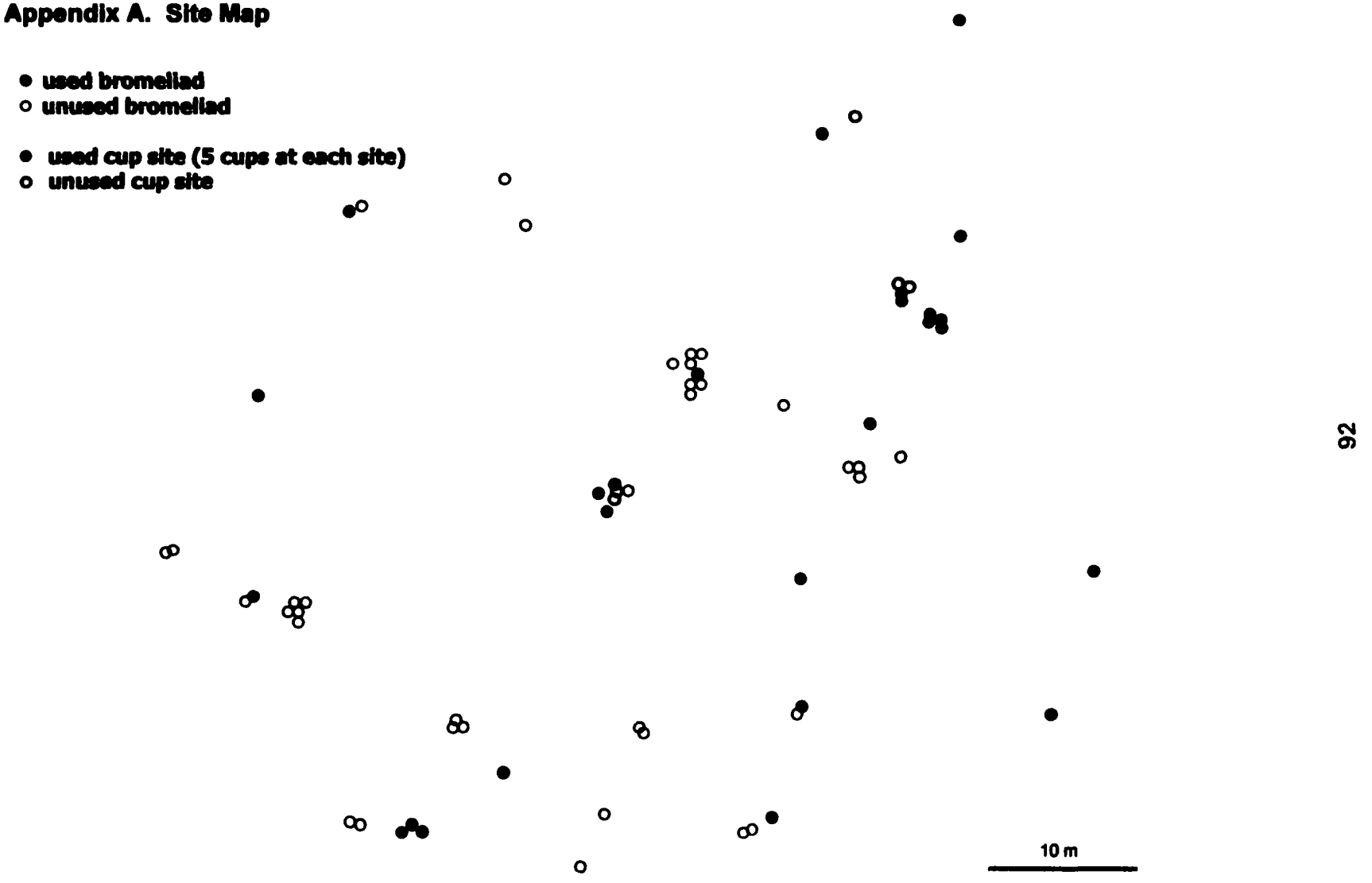


Table 1. Relatedness of ovipositing parents to resident tadpoles already in nurseries during 252 sub-clutch deposition events that did not involve male recruitments ($n_1 = 14$ females, $n_2 = 12$ males).

	Sub-clutches deposited in sites containing own offspring
Females	82.5% ± 27.2%
Males	81.3% ± 35.5%

Table 2. Relatedness of ovipositing parents to resident tadpoles already in nurseries during 16 male recruitment events. These 58 sub-clutch depositions involved seven females and nine males ($n = 11$ male/female pairs).

	Sub-clutches deposited in sites containing own offspring
Females	71.8% ± 37.1%
Males	12.3% ± 26.0%

CAPTIONS

Figure 1. Mating success (indicated by number of matings) for males as a function of time. Analysis was limited to males with more than two matings ($r^2_{adj}=0.24$, $F_{1,24}=9.03$, $P=0.006$, $n=26$ males)

Figure 2. Mating success (indicated by number of matings) for females as a function of time. Analysis was limited to females with more than two matings ($r^2_{adj}=0.61$, $F_{1,26}=42.78$, $P<0.001$, $n=28$ females)

Figure 3. The number of females that males mated with at clumped and isolated sites. Males at clumped sites (median = 3, $n=13$) mated with a greater diversity of females than did males at isolated sites (median = 1, $n=15$) (Mann-Whitney $U=156$, $P<0.001$). Symbol (•) represents an outlier.

Figure 4. The number of males that females mated with at clumped and isolated sites. Females at clumped sites (median = 4, $n=11$) mated with a greater diversity of males than did females at isolated sites (median = 1, $n=15$) (Mann-Whitney $U=153.5$, $P<0.001$). Symbol (•) represents an outlier.

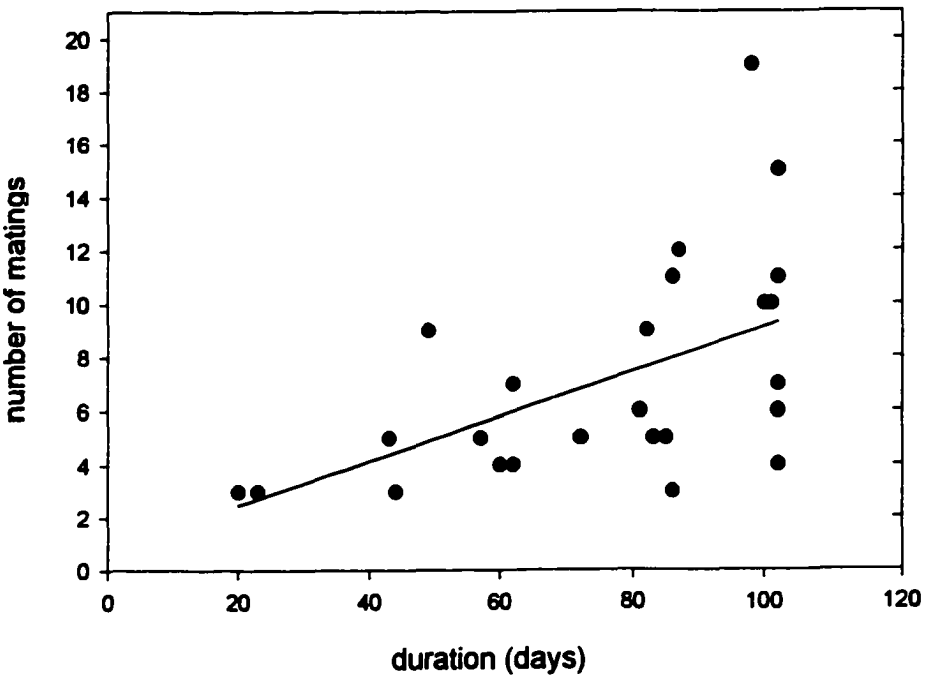

number of matings
duration (days)
0
2
4
6
8
10
12
14
16
18
20
0
20
40
60
80
100
120

FIGURE 1

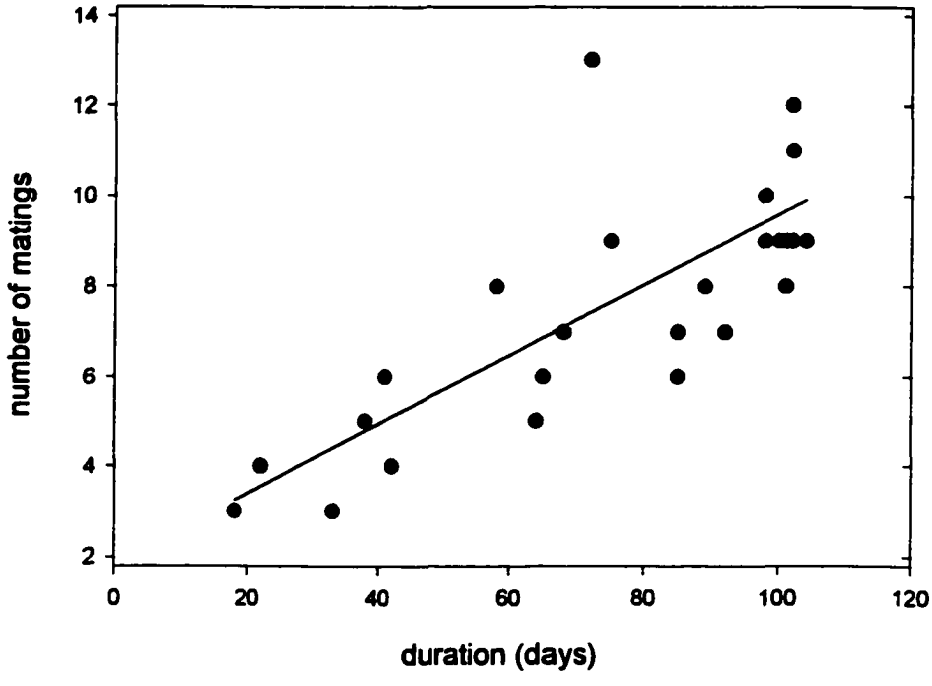

number of matings
14
12
10
8
6
4
2
0
20
40
60
80
100
120
duration (days)

FIGURE 2

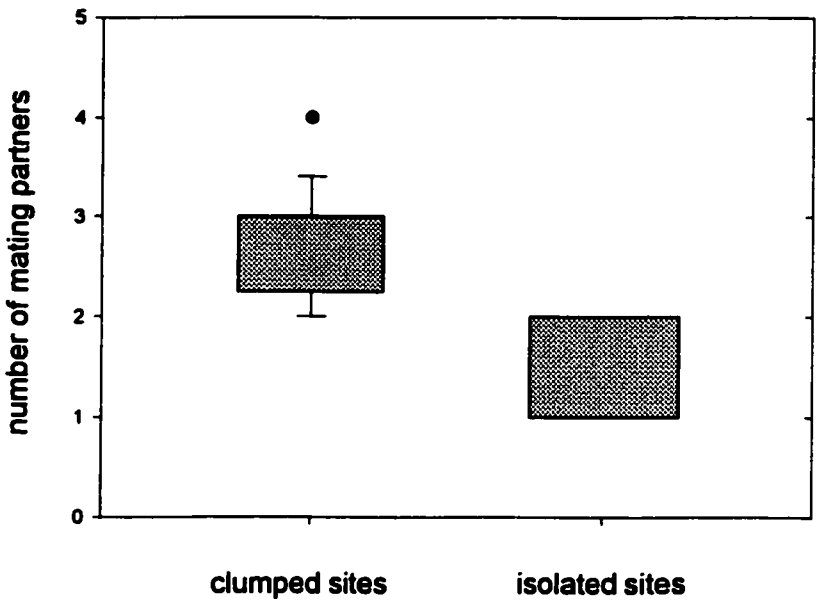

number of mating partners
5
4
3
2
1
0
clumped sites
isolated sites

FIGURE 3

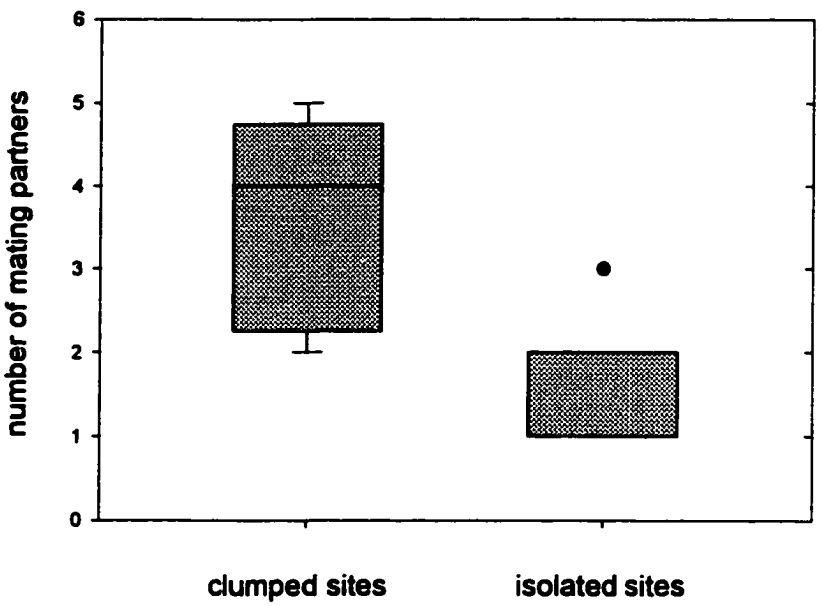

number of mating partners
6
5
4
3
2
1
0
clumped sites
isolated sites

FIGURE 4