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Author(s): Matthew R. Pintar and William J. Resetarits Jr
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Out with the Old, in with the New: Oviposition Preference Matches Larval Success in Cope's Gray Treefrog, *Hyla chrysoscelis*

MATTHEW R. PINTAR[1] AND WILLIAM J. RESETARITS JR

Department of Biology, University of Mississippi, University, Mississippi 38677 USA

ABSTRACT—**Oviposition site selection by breeding amphibians determines larval habitat for their offspring. Many amphibians breed in temporary ponds, which vary in hydroperiod and levels of competition, predation, and resource abundance. Newly filled ponds have fewer competitors and predators and more periphyton and phytoplankton, which are more palatable food sources for anuran larvae. We tested for oviposition site preference between old and new water pools in *Hyla chrysoscelis*, a species known to have better larval performance in newly filled pools. Consistent with larval performance and the observation that anurans often breed in ponds immediately after they fill, *H. chrysoscelis* selectively oviposited in new pools. Conductivity was significantly lower and dissolved oxygen significantly higher in new versus old pools, and these may be cues of pond age and productivity, respectively. We demonstrate that adult oviposition site selection preferences in our study match larval performance differences seen in previous work and that this preference is not simply for newly filled ponds but for ponds with a recent influx of water. These results further suggest novel ways for land managers to increase amphibian populations. Draining ponds will increase their attractiveness to breeding females who simultaneously avoid fish and choose new water.**

During oviposition, a female selects habitat for her offspring, determining the quality of the offspring's environment and dictating larval performance and adult fitness (Resetarits and Wilbur, 1989). Because habitat selection is a response to variation in habitat quality, adults would be expected to choose oviposition sites that maximize larval performance (Fretwell and Lucas, 1970; Morris 2003). The relationship between oviposition site preference and larval performance has been well studied in insects (Jaenike, 1978; Mayhew, 1997), where offspring have better survival on preferred plant species and females lay more eggs on these preferred plants (Gripenberg et al., 2010). Work on insects and amphibians shows that optimal habitat matching results in better larval performance and survival (Rieger et al., 2004). Oviposition in poor larval habitats can greatly reduce or completely eliminate the lifetime reproductive output of some taxa (Spencer et al., 2002).

Earlier breeding by anuran amphibians at temporary ponds provides offspring with an advantage over later breeders. Filling releases nutrients from dead plant and animal matter already in the pond, which produce early successional periphyton and phytoplankton blooms more palatable for anuran larvae than later successional filamentous algae and macrophytes (Seale, 1980). Anurans have higher larval survival, shorter larval periods, and larger metamorphic size in recently filled ponds, and early arriving individuals can shape community structure via priority effects (Wilbur and Alford, 1985). As hydroperiod increases and ponds age, the abundance of predators and competitors increases (Wellborn et al., 1996), and resources decline for larval anurans as they are stored in the biomass of plants and animals, which then can be exported from a pond (Wilbur, 1997; Kraus and Vonesh, 2012). Therefore, the "priority advantage" derives from reduced risk of death via desiccation and predation, increased resource availability (Wilbur, 1987), and decreased competition (Alford and Wilbur, 1985).

Amphibians select breeding sites based on a variety of site characteristics, including biotic factors, such as the presence of predators and competitors (Resetarits and Wilbur, 1989), and abiotic factors such as water depth, sediment depth (Rudolf and Rödel, 2005), and canopy coverage (Binckley and Resetarits, 2007). Other chemical differences between ponds that may relate to pond age, such as conductivity, dissolved oxygen (DO), and pH, can correlate with patterns of pond use (Vos and Stumpel, 1996; van der Voet and Stumpel, 1998). Therefore, breeding anurans likely respond to the chemical cue(s) (or lack thereof) of new water in recently filled ponds and select these sites as their preferred breeding habitat. Timing of oviposition and selection of breeding sites may interact as breeding subsides later in the summer, and many temporary ponds begin to dry. Temporary ponds provide critical breeding habitat for amphibians, and their use of temporary ponds is an important evolutionary adaptation for maintaining complex life cycles (Wassersug, 1975).

Although many anurans often breed in ponds immediately after they fill (Woodward, 1983), their "preference" for newly filled ponds should not be axiomatic in amphibian ecology (Duellman and Trueb, 1986), as the preference for newly filled ponds has not been experimentally demonstrated. In the southeastern United States *Hyla chrysoscelis* (Cope's Gray Treefrog; Cope, 1880) breeds April through August in an array of pond types, including experimental mesocosms, and has greater survival, grows larger, and reaches metamorphosis faster in recently filled ponds (Wilbur and Alford, 1985). They can metamorphose in as little as one month, and juvenile growth can be partitioned between their aquatic and terrestrial stages, enabling them to take advantage of temporary ponds while also metamorphosing before they dry (Wilbur and Collins, 1973). To determine whether oviposition site preference matches previously observed larval performance differences in *H. chrysoscelis*, we investigated the effects of pond water age and resulting chemical characteristics on oviposition site preference of a natural population of *H. chrysoscelis* in a landscape of experimental mesocosms.

MATERIALS AND METHODS

Experimental ponds (plastic wading pools: 1-m diameter; 110 L), arranged in a 4 × 6 rectangular array (1 m between pool

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[1] Corresponding Author. Email: mpintar@go.olemiss.edu

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TABLE 1. Water chemistry and ANOVA results for differences between new and old water pools. Mean pH was calculated by converting each pool's pH to hydrogen ion concentrations, calculating the arithmetic average of all pools in each treatment, and converting back to pH.

					New water		Old water			
	SS	df	*F*	*P*	Mean	SE	Mean	SE	Well	Rain
Conductivity (μS/cm)	4.48	1	721.37	<0.0001	25.01	0.47	59.43	1.56	18.8	7.1
Residuals	0.14	22								
DO (mg/L)	0.09	1	5.37	0.0301	5.24	0.20	4.64	0.17	6.68	7.74
Residuals	0.35	22								
pH	0.17	1	0.91	0.3535	6.67	0.10	6.72	0.16	5.49	5.82
Block	1.76	5	1.92	0.1433						
Residuals	3.11	17								
Temperature (°C)	0.00	1	0.00	0.9957	34.17	0.32	34.17	0.30	18.6	20.1
Block	0.01	5	3.47	0.0242						
Residuals	0.01	17								

edges) were established on 31 July 2014 at the University of Mississippi Field Station (UMFS) near Oxford, Mississippi, in a field 38 m north and 45 m west of the nearest ponds, which contained fish. Two treatments, old water (old) and new water (new) (12 replicates each), were arranged in a block design with rows ($N = 6$, south–north toward forest edge) as blocks. We randomized the treatment of the first pool in the first block and systematically alternated treatment between pools by row and column; thus, no pools of the same treatment were adjacent. Pools were filled with water from a nearby well and had 0.5 kg of leaf litter (primarily *Quercus* spp.) added at the time of filling. Pools were not inoculated with pond water to mimic newly filled ponds with no preexisting aquatic biota; uninoculated ponds nonetheless rapidly develop algal, phytoplankton, and zooplankton assemblages (Louette and De Meester, 2005). Window screening (1.3 mm^2, 1.13 mm opening) was used to cover the pools and depressed below the water surface to segregate frog eggs from the leaf litter and enable collection. Once every seven days for four weeks, beginning on 7 August, nearly all water ($> 95\%$) in the new pools was bailed using a bucket. Water was bailed from above the screen such that no leaf litter (placed below the screen) was lost during the bailing process. New pools were refilled, and old pools were topped off, both with well water, to maintain equal volumes. Pools were checked daily for frog eggs, which were collected, counted, and placed in nearby fishless ponds. The experiment was terminated on 4 September 2014. We measured pool temperature, conductivity, and pH with a YSI 63/25 FT meter (YSI, Inc., Yellow Springs, OH), dissolved oxygen (DO) with a YSI 550 DO meter, and analyzed all variables with separate ANOVAs on log-transformed data (except pH). We also measured the above variables for well water and rainwater (from the previous night) on 28 and 31 August, respectively (Table 1). We examined the effects of treatment and block on mean total eggs using square-root transformed data and ANOVA. Our primary response variable, total number of eggs laid per pool during the course of the experiment is the most ecologically meaningful way of analyzing our data and does not inflate statistical power relative to using number of egg masses laid. Although neither eggs nor egg masses are independent pieces of information on treatment effects, egg counts within experimental units are independent estimates of treatment effects in unconstrained natural populations. All analyses were conducted using type III SS ($\alpha = 0.05$) and R v. 3.2.2 (R Core Team, 2015). All descriptive statistics are reported as mean ± SE.

RESULTS

Hyla chrysoscelis laid a total of 6,554 eggs in new pools (655.4 ± 203.2) and 44 in old pools (4.4 ± 4.4) ($F_{1,18} = 15.22$, $P = 0.001$, Fig. 1). We estimate this represents the output from eight to 11 females based on the spatial and temporal distributions of eggs and average clutch size. Because the experiment was conducted late in the season, breeding activity was limited, but the difference in response was nonetheless definitive. All eggs in old pools were laid in one pool on 8 August, the first rainy night during the experiment, whereas oviposition in new pools occurred on five of the six nights (Fig. 1; also 3 September) when more than a trace of precipitation was recorded at the University and Abbeville, Mississippi, weather stations (National Oceanic and Atmospheric Administration, 2014). Seven of the 10 new pools included in the analysis received eggs, and two of these pools received eggs on multiple nights. No oviposition by *H. chrysoscelis* was observed anywhere at UMFS after 31 August. The northernmost row (two replicates of each treatment) received zero eggs and was eliminated from the egg analysis, whereas the effect of block on number of eggs, conductivity, and DO was not significant ($P > 0.25$) and was included in the error term. Water changes significantly decreased pool conductivity and increased DO but did not significantly affect pH and temperature (Table 1).

All data are accessible in Figshare http://dx.doi.org/10.6084/m9.figshare.3505688.

DISCUSSION

Preference-performance theory predicts that when there are larval performance differences between patches of varying quality, there should also be matching oviposition site preference differences among these patches. Many amphibians have been observed breeding in ponds soon after they fill, and one of these species, *H. chrysoscelis*, is known to have better larval performance in newer ponds (Wilbur and Alford, 1985). Given these larval performance differences in *H. chrysoscelis*, we would expect to observe matching adult oviposition site preferences such that individuals are able to maximize their expected fitness.

In our experiment, *H. chrysoscelis* selected oviposition sites with new water over those with old water only eight days after pools were established. Differences of six days in the timing of arrival of species can have important effects on species interactions and community structure that persist through the summer (Wilbur and Alford, 1985). Our study was conducted in late summer, when most females ovipositing in our array had

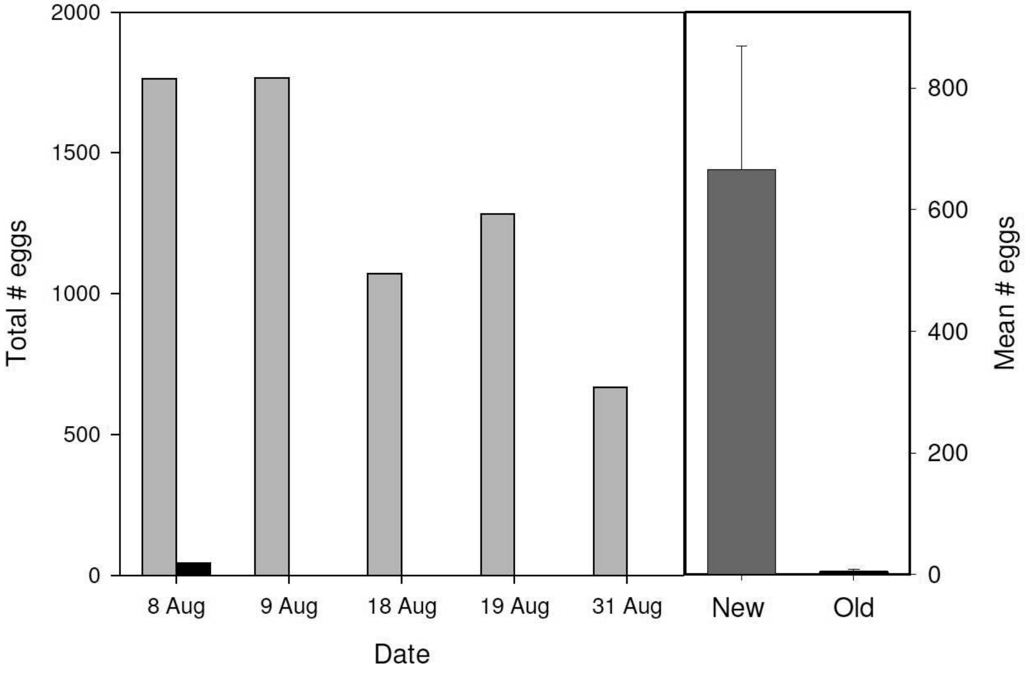

FIG. 1. Total number of eggs per treatment (gray = New, black = Old) for each night oviposition occurred, and mean (± SE) number of eggs per New (dark gray) and Old (black) pools over the duration of the experiment.

likely already laid a clutch earlier in the summer or were younger females that recently reached maturity. At this time, many of the fishless ponds at our study site had begun to dry, and larvae of many amphibians that bred earlier in the year were completing metamorphosis. The choice of oviposition site by females at this point in the year is likely influenced by factors such as predation risk, resource availability, expected hydroperiod, and water temperature. Because larvae do not overwinter, the emphasis remains on larval survival, but growth rate takes on increased importance as they need to complete metamorphosis and prepare for winter.

Both conductivity and DO differed significantly between old and new pools. Female *H. chrysoscelis* may be responding to total dissolved solids, particularly dissolved organics that have leached from dead organic matter or have been metabolized by other organisms in older ponds, or responding to increased DO, which would indicate higher algal productivity. Conductivity increases as temporary ponds age and dry, concentrating more dissolved solids in a smaller volume, and it also is higher in ponds with high nutrient levels regardless of pond age or hydroperiod. Therefore, high conductivity can be an ambiguous indicator of habitat quality, indicating both higher resource availability and desiccation risk (Spencer and Blaustein, 2001). Dissolved oxygen should be a more reliable indicator of algal production relative to animal metabolism, but because DO was undersaturated in both new and old pools, it may be difficult to relate to productivity. Although we show that *H. chrysoscelis* selects new pools with low conductivity/higher DO, as well as pools with larger volumes but equal surface area (Pintar and Resetarits, unpubl. data), they do not respond to increased nutrient levels directly (Binckley and Resetarits, 2008). Females may respond negatively to dissolved solids (conductivity) as an indicator of increased desiccation risk and lower habitat quality and use DO as a positive indicator of productivity and enhanced larval growth. Of course, other cues, perhaps emanating directly from biota, may be used for assessment.

Although many amphibians often are found in new ponds and have better larval performance in these habitats, oviposition preference for newly filled ponds has not been demonstrated (Wilbur, 1980; Alford and Wilbur, 1985; Wilbur and Alford 1985; Morin 1990; Murphy, 2003; Church, 2008). In a similar study, ovipositing Túngara Frogs, *Physalaemus pustulosus*, in Panama did not prefer newly filled ponds two, four, and eight weeks younger than paired old water ponds (Fegraus and Marsh, 2000). Larval growth and survival did not differ between old and new ponds, however, suggesting no selective advantage to breed in new ponds. In both newly created habitats (typically via disturbances) or newly available habitats (e.g., seasonal colonization of temperate breeding habitats by migratory birds), benefits gained from early arrival at new habitats stems from being able to secure optimal breeding sites, avoid predation, and reduce competition (Alve, 1999; Kokko, 1999). These priority effects occur in many systems and allow early colonizers to gain advantages over later colonizers (Alford and Wilbur, 1985; Facelli and Facelli, 1993; Shorrocks and Bingley, 1994; Almany, 2003).

Although many anurans are observed to breed in recently filled ponds, we demonstrate that *H. chrysoscelis* is selecting ponds with new water, not simply ponds that have recently filled, because our pools never really dry. Choosing recently replenished ponds does not necessarily place them in fishless habitats, as would typically be the case with newly filled ponds, therefore explaining the strong direct avoidance of ponds containing fish (Binckley and Resetarits, 2003). *Hyla chrysoscelis* appear to directly assess larval habitat in two stages: one assessment of potential productivity/desiccation risk; and one of predation risk. Our results provide evidence for habitat selection behaviors and oviposition site preferences of adult anurans that match the performance of larvae in those predator-free habitats. Adult *H. chrysoscelis* in our experiment oviposited in new ponds, and larvae in previous studies performed better in new ponds than in old ponds (Wilbur and Alford, 1985). Ovipositing female frogs select habitats most beneficial to their offspring to maximize fitness (Resetarits, 1996), but these decisions can also affect population growth rates, abundance and persistence of individual species, and the assembly of pond communities. The strategic drying of ponds can be a useful tool for improving amphibian breeding habitat quality and managing populations by both removing fish (Knapp et al., 2007) and resetting the overall community structure, including in fishless ponds. Pond drying provides optimal breeding habitat for amphibians that select not only fishless ponds but also those with new water.

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