

Effect of body size, age and timing of breeding on clutch and egg size of female Eastern Gray Treefrogs, *Hyla versicolor*

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Abstract. Information on how organisms allocate resources to reproduction is critical for understanding population dynamics. We collected clutch size (fecundity) and egg size data of female Eastern Gray Treefrogs, *Hyla versicolor*, and examined whether observed patterns of resource allocation are best explained by expectations arising from life history theory or by expected survival and growth benefits of breeding earlier. Female *Hyla versicolor* showed high between-individual variation in clutch and egg size. We did not observe maternal allocation trade-offs (size vs number; growth vs reproduction) predicted from life history theory, which we attribute to the large between-female variation in resource availability, and the low survival and post-maturity growth rate observed in the study population. Rather, clutches are larger at the beginning of the breeding season, and this variation in reproductive investment aligns with seasonal variation in ecological factors affecting offspring growth and survival.

Keywords: allocation, life history, reproduction, trade-offs.

Introduction

Species vary widely in their life history characteristics, including how often they reproduce, how many offspring they have, and how heavily each offspring is provisioned (Kaplan and Salthe, 1979; Fabian and Flatt, 2012). Variation in these traits is also present among different populations of the same species (Berven, 1982), and among and within females in the same population (Kaplan, 1980; Crump, 1984). Since both the number and size of eggs an organism produces has important consequences for individual fitness as well as population viability, it is of interest to identify the factors that influence reproductive output.

In amphibians and other ectothermic vertebrates, female body size is frequently positively correlated with fecundity (Tilley, 1968; Morita and Takashima, 1998). Amphibians also

show indeterminate growth; their body continues to grow after maturation, though at decreasing rates (Gibbons and McCarthy, 1984; Ryser, 1988). Age is thus often – but not always – correlated with body size (Halliday and Verrell, 1988, and references therein). It may thus be beneficial to delay reproduction to first invest in greater body size, because larger bodies can hold more eggs. Yet, it takes time to grow, and optimal body size depends on the reproductive benefits of being large and the costs of becoming large, particularly the probability of surviving to first reproduction (review in Blanckenhorn, 2000). Life history theory predicts a number of trade-offs between resource allocation for reproduction vs. growth, survival, and future reproduction (Stearns, 1992; Nilsson and Svensson, 1996). Knowledge of average lifespan, as well as amount of post-maturity growth

and the degree of increased fecundity derived from additional growth are of paramount importance for discussions about current and future benefits of resource allocation strategies.

In species with a breeding season that spans several weeks or months, such as prolonged breeding anurans, the timing of reproduction may also be informative for evaluating resource allocation patterns. Determining when a female reproduces within a given breeding season may provide insight into the energy budget that is required to prepare a clutch, and into among-female variation in quality (foraging or metabolic efficiency). In temperate frog species, for example, early breeding would suggest that resources were accumulated before the onset of hibernation in the preceding year. Asynchronous timing of breeding across a longer time period on the other hand would suggest that some females had accumulated required resources before hibernation, while others required additional foraging time (Ritke et al., 1990). Early-breeding females should also be more likely to acquire additional resources to lay a second clutch in the same breeding season, thus increasing their overall reproductive output (Denton and Beebee, 1996; Cadeddu and Castellano, 2012).

Resource availability and energy budget of the parental individuals may not be the only factors selecting for when to breed or how much to invest in reproduction. Environmental conditions affecting offspring survival may also play a role. For example, there are multiple benefits of early breeding for frogs (summarized by Loman, 2009) that should select for investing more into clutches that are laid earlier in the season (i.e., larger clutches and/or larger eggs). By breeding early females may increase the survival chances of their offspring: competition from other tadpoles, as well as predation risk, are lower for early-hatching tadpoles (Loman, 2001; Matsushima and Kawata, 2005). Early-hatching tadpoles likely also metamorphose earlier, giving them more time to forage

as froglets. This may allow them to reach winter hibernation at a larger size (Altwegg and Reyer, 2003), which may in turn increase survival, and/or allow them to reach sexual maturity earlier or at a larger size.

Here we explore the factors affecting clutch and egg size of female Eastern Gray Treefrogs, *Hyla versicolor*, a common North American frog species with a breeding season that spans several weeks (i.e., prolonged breeder). Given the aforementioned commonly observed patterns in ectothermic vertebrates with indeterminate growth, we predicted that larger females would have more and/or larger eggs. Following life history theory, we also predicted to see an increase of investment with age (i.e., declining chance of survival), as well as a trade-off between the size and number of eggs in a clutch. Given the potential benefits of early breeding for frogs (Loman, 2009), we further predicted that females breeding earlier in the season would invest heavier in reproduction, by laying more and/or larger eggs, potentially at the cost of retaining less residual body condition.

Methods

Study species and study site

We studied the reproductive ecology of Eastern Gray Treefrogs (*Hyla versicolor*) at a pond adjacent to the University of Wisconsin–Milwaukee Field Station (Saukville, WI, USA). This is a pond of ca. 1.3 ha size, completely surrounded by woodland. Females were collected by teams of 2–4 people using headlamps to search for pairs found in amplexus. Searching occurred between 2100–0100 hours on nights in which weather conditions were amenable for frog reproductive activity (i.e., temperature and rainfall intensity allowing for breeding choruses to form). Sampling was not randomized; rather, we attempted to find as many pairs as possible. Pairs were transported to the University of Wisconsin–Milwaukee, where data collection took place. After data collection (spawning, size measurements), both males and females were released at the collection site.

Data collection

Breeding activity. We sampled female breeding activity over 3 seasons (May–July, 2009–2010 and 2016). In 2009 and 2010 we individually marked every female we collected by implanting a fluorescent alpha-numeric tag beneath the skin of the ventral side of the thigh of the right hind limb (VI Alpha Tags, size 1.0 × 2.5 mm, 0.1 mm thick; Northwest

Marine Technology Inc., Shaw Island, WA, USA). This allowed us to assess whether some females bred repeatedly within the same or consecutive years, and whether their body size or clutch measures changed over time. Female arrival is not uniform across the season, and on dates we collected a large number of females we haphazardly selected 5-8 individuals from which to measure clutch data. All females included in this study were collected from pairs in amplexus at the breeding pond. We collected them before midnight, which should be at least 2 hrs before they start laying eggs (Ritke et al., 1990). Still, we cannot preclude the possibility that females could have deposited some of their eggs before we captured them.

Clutch and egg size. To obtain clutch and egg size data, each pair was placed in a spawning container (30 × 15 cm plastic bin with lid that contained 2 liters of aged tap water), and kept in a quiet dark place until they laid eggs (generally overnight). After spawning, we photographed the entire clutch with a digital camera (Canon PowerShot A75 or Canon EOS digital Rebel; Canon Inc., Tokyo, Japan). We then manually counted the total number of eggs laid by each female (in 2009, 2010, and 2016). In 2016, we haphazardly selected 10 eggs, which we transferred to a petri dish and photographed with a digital camera (Moticam; Kowloon, Hong Kong) mounted on a stereomicroscope (Leica EZ4D; Leica Microsystems, Wetzlar, Germany) with 45× magnification. A scale bar was visible in each photograph. We used ImageJ version 1.48 (National Institutes of Health, Bethesda, MD, USA) to measure yolk diameter (best fit across the main body of each yolk). We measured the size (yolk diameter in mm) of 510 eggs from 51 clutches (i.e., 10 eggs per clutch).

Body size and condition. After oviposition, females were weighed (post-spawn body mass) to the nearest 0.1 g with a digital scale (Ohaus CS200; Parsippany, NJ, USA), and we also measured their snout-vent length (SVL) (to nearest 0.1 mm) with a caliper. Since level of hydration can affect weight measurements, we encouraged the frogs to evacuate their bladder by gently padding them between pieces of paper towel before each weight measurement. We calculated body condition as the residuals of a regression of log-transformed SVL on log-transformed post-spawning body mass (Băncilă et al., 2010). Females with positive residuals were considered in good body condition because they were heavier than expected from their body length.

Age determination. To estimate the age of female treefrogs, we used skeletochronology. This method has been successfully used with frog species from a range of families, including treefrogs (Friedl and Klump, 1997; Cadeddu, Giacoma and Castellano, 2012). We removed the last two phalanges of up to two toes of each female, and preserved them in a 70% ethanol solution. For skeletochronological analysis, we followed the procedure described by McCreary et al. (2008). Briefly, preserved phalanges were cleaned of surrounding tissues and decalcified in Cal-Ex II. In preparation for paraffin infiltration and embedding, phalanges were dehydrated in 95% and then 100% ETOH, followed by 100% Shandon Xylene Substitute (Isoalka-

nes C9-12). Phalanx cross sections were obtained with a microtome at 16 mm, and mounted on microscope slides. After paraffin was removed using reverse Xylene Substitute and ETOH bath series, phalanges were stained with Harris' hematoxylin. For each section, two observers counted independently the number of lines of arrested growth (LAGs) under a light microscope and later compared results. Dubious cases were discounted. The most peripheral edge of the cross sections was counted as an additional LAG because the specimens were collected during the breeding seasons after emergence from hibernation (Rogers and Harvey, 1994).

Statistical analyses

Female age and body size. To explore whether age was related to body length (as is predicted for organisms with indeterminate growth such as anurans), we used a linear mixed-effect model, REML method, with body length as the dependent variable, age as the independent covariate, and the year of study as random categorical factor. We recaptured some females ($n = 7$) in consecutive years, which allowed us to investigate how female and clutch properties change over time. We did so using paired t -tests.

Variation in clutch size. To explore factors affecting variation in clutch size, we used a linear mixed-effect model, REML method, in which the number of eggs in a clutch was the dependent variable, and female age, body length, body condition, and oviposition date were independent covariates. The year of study and female ID were entered as random categorical factors.

We recaptured some females ($n = 13$) within the same year (2010). This allowed us to investigate the effect that repeat breeding has on clutch size and female condition, and to compare if there are differences between single and multi-clutch females. We did so using ANOVAs.

Variation in egg size. To explore factors affecting variation in egg size, we used ordinary least squares regression. Here, yolk diameter was the dependent variable, and female body length, female body condition, clutch size, and oviposition date were the independent covariates. Clutch size was included to test for a trade-off between the two components of female reproductive investment (i.e., size-fecundity trade-off). We were unable to analyze the effects of female age on egg size due to the mismatch of collection years between the two variables (female age data from 2009 and 2010, and egg size data from 2016).

To determine if some females produced clutches with egg sizes of greater variability than others, we calculated the coefficient of variation ($CV = SD/mean$) for the egg diameter for each female. Then we used ordinary least squares regression to investigate potential factors affecting intra-clutch variability ($CV_{egg\ size}$). Here, $CV_{egg\ size}$ was the dependent variable, and female body length, female body condition, clutch size, and oviposition date were the independent covariates. This analysis was carried out only on the 2016 sample (the only year for which we had egg size data).

All statistical analyses were carried out using JMP 11 software (SAS Institute Inc., Cary, NC, USA). We adopt a $P < 0.05$ threshold for statistical significance, but provide exact P -values for each test.

Results

Descriptive statistics for the 215 females (71 in 2009, 93 in 2010, and 51 in 2016) measured in the study are shown in Table 1.

Female age and overall lifespan

Skeletochronology was carried out successfully on 57 adult females (70%; 16 of 26 in 2009, and 41 of 56 in 2010), a success rate comparable to other studies (i.e., 58% in Cadeddu et al., 2012). Female age estimates ranged between 1-3 years, demonstrating that this species can reach sexual maturity at one year of age.

Overall lifespan is quite short. Our sample of the breeding populations contained many young, one-year old females (64.3%). The proportion of breeding females decreased with age: 25% of breeding females were two year old, and

Table 1. Descriptive statistics of *Hyla versicolor* female and clutch metrics.

	mean	SD	range
Body length (SVL) [mm]	46.4	3.4	38.5-55.5
Post-oviposition mass [g]	8.6	1.9	5.1-14.7
Body condition*	$1.2 e^{-15}$	0.12	-0.28 ± 0.39
Age [yrs]	1.47	0.68	1-3
Mean clutch size [no. eggs]	959	421	227-2217
Mean egg diameter [mm]	1.42	0.14	1.12-1.69

*calculated from the mean residuals of a regression of log-transformed SVL on log-transformed post-spawning body mass, and hence expected to be very close to 0.

10.7% were three year old. This low age distribution is in line with our low recapture rate. During 2009 and 2010 we marked a total of 193 females, but recaptured only 16 females (14 females after one year later, and 2 females after two years).

As expected for a species with indeterminate growth, older females were on average larger than younger ones (Fig. 1A; $F_{1,54} = 5.35$, $P = 0.04$; model coefficient \pm SE: 1.67 ± 0.71). However, age-related differences in body length were very small (average difference of 2 mm). The size at which females reach maturity also varied greatly; SVL of 1-year old females ($n = 36$) ranged from 38.9 to 52.3 mm. Having recaptured some females in consecutive years afforded a second way of assessing post-maturity growth. Between years, SVL did significantly increase (Fig. 1B; paired t -test: $t = 2.98$; $df = 6$, $P = 0.02$; mean difference 2.4 mm; comparable to average yearly increase in SVL derived from female age data, which was 2 mm), but post-spawn condition did not change across years ($t = -1.04$; $df = 6$, $P = 0.34$). Importantly, mean clutch size did not change between years ($t = -0.11$; $df = 6$, $P = 0.92$), suggesting that the average 2 mm increase in body length, while statistically significant, is biologically negligible in terms of increasing fecundity.

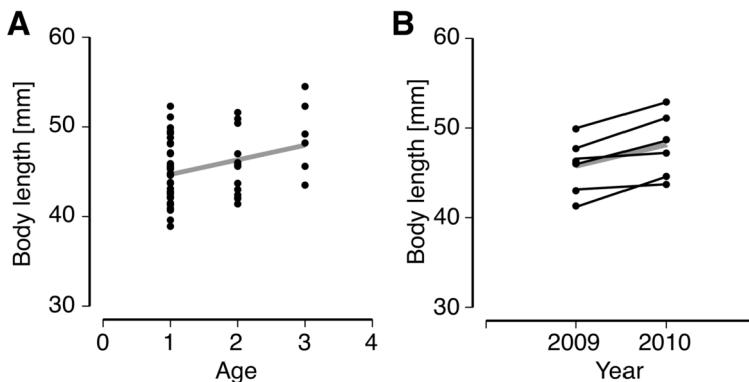


Figure 1. Lifespan, age and between-year growth of female *Hyla versicolor*. (A) Age distribution of females in the study population, and the relationship between age and body length. (B) Change in body length of the 7 females that were recaptured in consecutive years. Gray lines indicate averages.

Table 2. Determinants of *Hyla versicolor* clutch size (no. eggs). In 2016 we did not collect data on age, so to enter age in the model we had to restrict the data set to 2009 + 2010 (bottom).

Covariates	Coefficient \pm SE	df	<i>F</i>	<i>P</i>
2009 + 2010 + 2016 data set [clutch size \sim Body Length + Body Condition + Oviposition Date + (Year)]				
Body length [mm]	37.8 \pm 6.3	1,124.3	36.4	<0.001
Body Condition	519.3 \pm 186.5	1,216.2	7.76	0.006
Oviposition date	-20.3 \pm 2.0	1,212.7	104.3	<0.001
2009 + 2010 data set [clutch size \sim Body Length + Body Condition + Oviposition Date + Age + (Year)]				
Body length [mm]	45.8 \pm 11.8	1,51.3	15.1	<0.001
Body Condition	738.9 \pm 385.2	1,51.7	3.68	0.06
Oviposition date	-28.8 \pm 4.6	1,51.4	39.9	<0.001
Age [yrs]	48.8 \pm 61.0	1,51.1	0.64	0.43

Between-female variation in clutch size

Clutch size can vary by an order of magnitude (range: 227–2217 eggs), with an among-female coefficient of variation (*CV*) of 42% (only first clutches of each female were counted). Controlling for year (entered as random term in the model), variation in clutch size was significantly influenced by body length and oviposition date (Table 2). Clutch size increased with female body length ($P < 0.001$; Fig. 2A) but decreased from the beginning to the end of the breeding season ($P < 0.001$; Fig. 2D). Post-spawn body condition was positively associated with clutch size, but whether the relationship reached statistical significance depended on sample size and the inclusion of age as a covariate ($P < 0.06$ or $P < 0.006$; Table 2, top vs bottom, Fig. 2B). Age had no significant effect on clutch size ($P = 0.43$; Fig. 2C, Table 2).

Single vs multi-clutch females

Some females laid more than one clutch during the same breeding season ($n = 13$ in 2010; 12 females laid two clutches, and one female three clutches). The between-clutch mean delay was 20 ± 4 days (range: 12–24 d). Laying two clutches significantly increased overall fecundity (ANOVA: $F_{1,87} = 65.6$, $P < 0.001$); females with two clutches laid on average 904 more eggs than females that had only one clutch, which is an increase of 48%. A female's first clutch was on average 2.8 times larger than her second one (mean of 1378 vs 487 eggs;

$F_{1,24} = 62.7$, $P < 0.001$). Between successive clutches, the condition of the females remained similar ($F_{1,24} = 1.74$, $P = 0.20$).

Multi-clutch females were neither significantly larger ($F_{1,87} = 3.34$, $P = 0.07$), nor in better condition ($F_{1,87} = 0.38$, $P = 0.54$). However, the first clutch of multi-clutch females was laid earlier in the breeding season ($F_{1,87} = 17.2$, $P < 0.001$). Eight-five percent of the females that bred more than once in a given season produced their first clutch during the first week (11/13) if not the first day (6/13) of the breeding season. Moreover, multi-clutch females had on average larger first clutches than single-clutch females (on average 383 eggs more; $F_{1,87} = 12.9$, $P < 0.001$). Female Gray Treefrogs release all eggs when ovipositing, i.e., they do not hold back a portion of the clutch to lay eggs with several males sequentially over several nights (GH, pers. obs., of 12 females allowed to spawn with 2 different males in two consecutive nights; no eggs were deposited in the second night). Thus, eggs from the first clutch did not contribute to the second clutch.

Between-female variation in egg size (yolk diameter)

Variation of individual eggs size was pronounced: the smallest and largest yolk measured during the study was 0.90 and 2.22 mm, respectively. The mean diameter of yolks from each clutch ranged from 1.12–1.69 mm (mean \pm SD = 1.4 ± 0.14 mm, $n = 51$). The mean

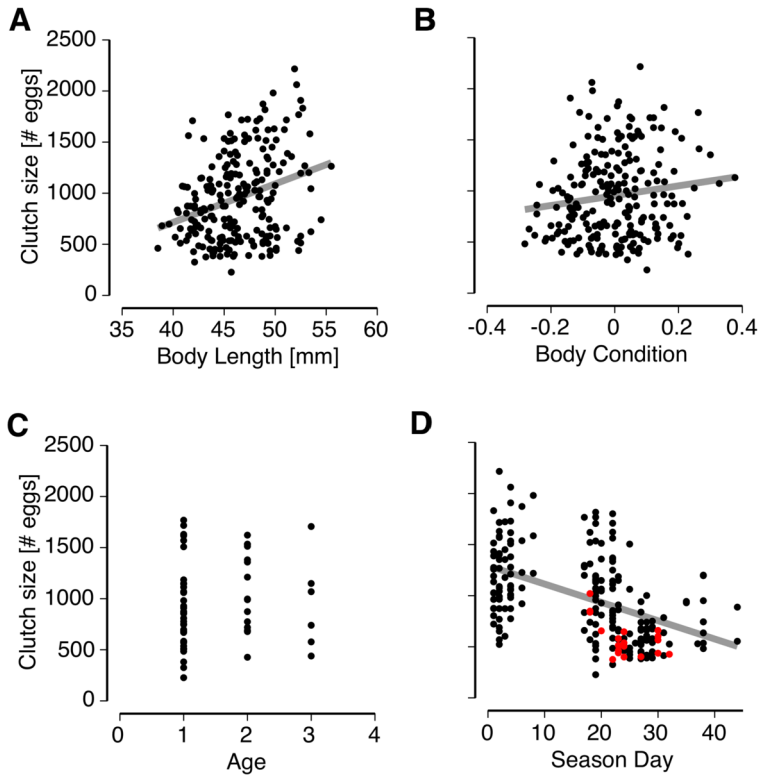


Figure 2. Determinants of clutch size (# eggs) in female *Hyla versicolor*. (A) Relationship between female body length and clutch size; (B) relationship between body condition and clutch size; (C) relationship between age and clutch size; (D) relationship between breeding season date and clutch size. Second and third clutches of repeat-breeding females are indicated as red symbols. Significant relationships are indicated by trendlines (see also Table 2).

between-female CV in average yolk diameter was 9.6%.

Variation in mean yolk diameter was not influenced by the body length or body condition of the females that laid them, nor the oviposition date or the clutch size (Table 3). There was also no trade-off between yolk diameter and egg number (Table 3).

Within-clutch variation in egg size

There was considerable within-clutch variation in egg size (yolk diameter). The mean and SD of the within-clutch coefficient of variation ($CV_{egg\ size}$) for yolk diameter was $9.9 \pm 3.4\%$, but some clutches had 5 times the variation than others (range: 3.3–18.0%). Within-clutch variation in yolk diameter was not influenced by female size, female condition, oviposition date or clutch size (Table 3).

Discussion

Female *Hyla versicolor* show high between-individual variation in clutch and egg size. We did not observe maternal allocation trade-offs predicted from life history theory. Rather, we suspect that variation in reproductive investment aligns with seasonal variation in ecological factors affecting offspring growth and survival, such as competition, predation, and foraging opportunity (Loman, 2009).

Factors affecting clutch and egg size

In line with comparative data from anurans with similar body size and life history, we observed that female *H. versicolor* that were larger and in better body condition have larger clutches (i.e., Gibbons and McCarthy, 1986; Tejedo, 1992; Cadeddu and Castellano, 2012). By contrast, we

Table 3. Determinants of *Hyla versicolor* mean egg size (top) and egg size variability ($CV_{\text{egg size}}$; bottom).

Covariates	Coefficient \pm SE	df	F	P
Mean egg size [mean egg size \sim Body Length + Body Condition + Clutch Size + Oviposition Date + (Year)]				
Body length	0.001 \pm 0.007	1,44	0.03	0.87
Body Condition	0.009 \pm 0.19	1,44	0.002	0.96
Clutch size [no. eggs]	5.7e ⁻⁵ \pm 5.6e ⁻⁵	1,44	0.98	0.33
Oviposition date	-0.002 \pm 0.002	1,44	1.11	0.30
$CV_{\text{egg size}}$ [CV of egg size \sim Body Length + Body Condition + Clutch Size + Oviposition Date]				
Body length	0.16 \pm 0.19	1,44	0.73	0.40
Body Condition	7.1 \pm 4.7	1,44	2.27	0.14
Clutch size [no. eggs]	-0.001 \pm 0.001	1,44	10.48	0.49
Oviposition date	0.05 \pm 0.06	1,44	0.75	0.39

did not observe an effect of body measures on egg size. The latter is observed in some anurans (Cadeddu and Castellano, 2012; Castellano, Cucco and Giacoma, 2004; Pereira and Maneyro, 2012), but less ubiquitous than the relationship between body size and egg number; a number of studies that did observe a positive correlation between body size and egg number failed to document a similar pattern with egg size (Lüddecke, 2002; Liao, Lu and Jehle, 2014; Lu et al., 2016).

Variation in clutch and egg size in *H. versicolor* did not show some of the patterns expected from life history theory, particularly commonly expected trade-offs. First, organisms may partition their reproductive effort between a few large or many small offspring (Smith and Fretwell, 1974; Lloyd, 1987), predicting a size vs number trade-off. While the egg size vs number trade-off is found when comparing *between* species in a wide range of taxa (Elgar, 1990; Blackburn, 1991; Pupin et al., 2010; in den Bosch and Bout, 1998), this trade-off is not always found when comparing *within* species (i.e., Tejedo, 1992; Roosenburg and Dunham, 1997; this study). However, a lack of negative association between number and size of eggs may occur if there is high variation in the amount of resources available to females (Van Noordwijk and de Jong, 1986). If the variation in the total investment (estimated by

$CV_{\text{clutch size}}$) is larger than variation in the allocation strategy (estimated by $CV_{\text{egg size}}$), then life-history theory does no longer predict a negative association between the number and the size of eggs. For our data set, the $CV_{\text{clutch size}}$ was 4 times higher than for $CV_{\text{egg size}}$, suggesting that the large between-female variation in resource availability may have obscured any potential egg size/number trade-off. Another line of evidence documenting great among-female variation in the amount of available resources to allocate in reproduction comes from the relationship between post-spawn body condition and clutch size. If all females had the same amount of resources, than those that invested more in reproduction (i.e., larger clutches) are expected to have lower post-spawning body condition. Yet, we found a positive correlation: some females were able to invest a lot in reproduction and maintain high body condition.

The second trade-off expected from life history theory is that reduced chance of survival should increase investment in reproduction. This suggests that older females should have more or larger eggs, which has been observed in *Rana temporaria* frogs (Gibbons and McCarthy, 1986). In our study we did not find that age was correlated with clutch or egg size. This lack of age-related effects on clutch parameters may be explained by the data on growth and survival we observed in our population. As may be expected from a species with indeterminate growth, age

was significantly but weakly correlated with body size (i.e., Friedl and Klump, 1997; but see Cadeddu and Castellano, 2012). Although age and recapture data congruently showed an average body length increase of about 2 mm per additional year of life after reaching maturity, this post-maturity increase pales in comparison to the staggering amount of growth that is possible during the first year of life (the largest one-year-old female was 52.3 mm, which is in the 95th percentile of the population). Temperate treefrogs generally start breeding at 2 years (Friedl and Klump, 1997; Cadeddu, Giacomina and Castellano, 2012; but see Cadeddu and Castellano, 2012), and can live to reach 6 years in the wild (see Friedl and Klump, 1997). By comparison, overall longevity was quite low in our population (most breeding females were one year old, and none was older than three), which may be offset by the comparatively early age at first reproduction. Low chance of survival and little additional growth after the first year of life should select against trade-offs between growth vs reproduction.

Intra-clutch variability in egg size

Hyla versicolor also shows considerable intra-clutch variability in egg size ($CV_{\text{egg size}}$) which is quite common for anurans (see also Dziminski and Alford, 2005; Cadeddu and Castellano, 2012; Yaeger and Gibbon, 2013). What determines the degree of within-clutch egg size variability is less clear. In our study we did not find a female trait that correlated with egg size variability, which is in line with data from another North American Hylid, *Pseudacris crucifer* (Crump, 1984). By contrast, in Red Eyed Tree Frogs, *Agalychnis callidryas*, smaller females had more variable clutches (Yaeger and Gibbons, 2013), and in Quacking frogs, *Crinia georgiana*, larger females had more variable clutches (Dziminski and Alford, 2005).

Two hypotheses have been proposed for why egg size may be variable within a clutch. The

bet-hedging hypothesis states that egg size variability is an adaptation for dealing with unpredictable environments (Crump, 1981; Dziminski, Vercoe and Roberts, 2009). To our knowledge this hypothesis has so far been tested most directly by comparing egg size variability between species that breed in temporary vs permanent ponds ($CV_{\text{egg size}}$ is predicted to be higher in temporary-pond breeders; Crump, 1981). For amphibian larvae, environmental predictability can be estimated in terms of hydroperiod, food availability and predator pressure (Crump, 1981). These factors tend to be more predictable in permanent ponds, allowing for selection for an optimal egg size: small in good quality environments, and large in poor quality environments (Smith and Fretwell, 1974; Crump, 1981). Temporary ponds, on the other hand, are less predictable (Crump, 1981). Here a mixed-size strategy provides higher fitness than producing just small eggs in poor quality ponds (where larger eggs would do better), but leads to increased parental fitness in a higher quality environment (Capinera, 1979). Adapted for temporal changes in within-clutch egg size variability across the breeding season, as examined here for *H. versicolor*, this hypothesis predicts that as the breeding season progresses, and increased competition from conspecific and heterospecific tadpoles, invertebrate predation pressure and danger of pond drying makes conditions more unpredictable, $CV_{\text{egg size}}$ should increase. We did not observe this in our study.

Alternatively, within-clutch variability may not be an adaptive strategy, but arise from physiological constraints that do not allow females to produce similar size eggs (Marshall, Bonduriansky and Bussiere, 2008). Female body size may restrict the ability to produce equal sized eggs, as well as variation of her physiological status or environmental fluctuations experienced during vitellogenesis (Fox and Czesak, 2000; Einum and Fleming, 2004). There may even be a physiological cost to producing offspring of uniform size (Marshall, Bonduriansky

and Bussiere, 2008). We also did not observe this in our study.

Seasonal variation in clutch and egg size

The factor that had the most consistent influence on clutch and egg size in female *H. versicolor* was oviposition date. Clutch size and egg size declined as the breeding season progressed. Across season declines in clutch size, like we observed in *H. versicolor*, has also been reported for Italian Treefrogs, *Hyla intermedia* (Cadeddu and Castellano, 2012), while American Green Treefrogs, *Hyla cinerea* (Gunzburger, 2006) and the Columbian treefrog *Hyla labialis* (Lüddecke, 2002) maintain similar levels of fecundity across the season. The breeding seasons of *H. intermedia* and Wisconsin *H. versicolor* are shorter than those of *H. cinerea* and *H. labialis* (1-2 vs 4-6 months) (Lüddecke, 2002; Gunzburger, 2006, Castellano et al., 2009), which may affect the type of selection acting on across-season changes in reproductive allocation strategies.

Some of the benefits of early breeding in frogs, such as reduced competition from other tadpoles and reduced predation risk for early-hatching tadpoles (Loman, 2001; Matsushima and Kawata, 2005) should be accrued independent of breeding season length. Other benefits may only be important for species with shorter breeding seasons where the need to have tadpoles complete development in time for hibernation/aestivation may select for a decline of investment towards later dates in the season. For example, early hatching tadpoles likely metamorphose earlier, which gives them more time to forage and to reach winter hibernation at a larger size (Altwegg and Reyer, 2003). This may in turn increase survival, and/or allow them to reach sexual maturity earlier or at a larger size. Variation in foraging time likely accounts for the variation in body size of same age individuals, as well as the range of variation in clutch sizes even early in the breeding season as observed in this and other studies (i.e., Cadeddu and Castellano, 2012).

Between-female variation in reproductive investment

Hyla versicolor shows considerable between-female variation in reproductive investment (i.e., high among-female coefficient of variation (CV) of clutch size and egg size, which is not uncommon among anurans (i.e., Cadeddu and Castellano, 2012). Several lines of evidence suggest that this variation is due to inherent quality differences between females. First, there was no trade-off between egg size and number, suggesting that females had different amounts of resources available to invest in reproduction. Second, there was a positive correlation between the maintained body condition and clutch size, indicating that some females can invest heavily in reproduction and still maintain good body condition. Finally, the average first-clutch size of repeatedly breeding females was larger than the average clutch size of one-time breeding females, yet post-spawn condition did not differ between these two types of females. Producing a second clutch thus did not come at the cost of allocating less energy or reducing fecundity in the first one. Rather, repeat breeding females were able to allocate more to reproduction initially, and to efficiently obtain additional resources for a second clutch. This suggests that it is not a question of sufficient foraging time that allows some females to produce a second clutch, but that those are inherently high quality individuals that had accumulated energy for an early clutch the year before, could afford to allocate a comparatively large amount of energy to reproduction, and then were still able to efficiently acquire resources that allowed the production of a second clutch.

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