POPULATION ECOLOGY

Density dependence in the terrestrial life history stage of two anurans

Elizabeth B. Harper · Raymond D. Semlitsch

Abstract Populations of species with complex life cycles have the potential to be regulated at multiple life history stages. However, research tends to focus on single stage density-dependence, which can lead to inaccurate conclusions about population regulation and subsequently hinder conservation efforts. In amphibians, many studies have demonstrated strong effects of larval density and have often assumed that populations are regulated at this life history stage. However, studies examining density regulation in the terrestrial stages are rare, and the functional relationships between terrestrial density and vital rates in amphibians are unknown. We determined the effects of population density on survival, growth and reproductive development in the terrestrial stage of two amphibians by raising juvenile wood frogs (*Rana sylvatica*) and American toads (*Bufo americanus*) at six densities in terrestrial enclosures. Density had strong negative effects on survival, growth and reproductive development in both species. We fitted a priori recruitment functions to describe the relationship between initial density and the density of survivors after one year, and determined the functional relationship between initial density and mass after one year. Animals raised at the lowest densities experienced growth and survival rates that were over twice as great as those raised at the highest density. All female wood frogs in the lowest density treatment showed signs of reproductive development, compared to only 6% in the highest density treatment. Female American toads reached minimum reproductive size only at low densities, and male wood frogs and American toads reached maturity only in the three lowest density treatments. Our results demonstrate that in the complex life cycle of amphibians, density in the terrestrial stage can reduce growth, survival and reproductive development and may play an important role in amphibian population regulation. We discuss the implications of these results for population regulation in complex life cycles and for amphibian conservation.

Keywords Population regulation · Amphibian · Reproductive development · Growth · Survival

Introduction

Populations of organisms with complex life cycles in which adult and juvenile stages occupy different habitat types have the potential to be regulated at one or multiple life history stages (Hellriegel 2000; Rodriguez 1988; Semlitsch et al. 1996; Wilbur 1980). Regulation occurs when the density of conspecifics reduces the growth, survival or fecundity of individuals, slowing the growth rate of the population. In stage-structured populations, changes in the vital rates of some stages often have a much greater effect on the growth rate of the population than others (Benton and Grant 1999). Small changes in the vital rates of these sensitive stages can reduce the population growth rate to a far greater degree than much larger changes in the vital rates of less sensitive stages.

Among organisms with complex life histories, stages with the greatest variability in vital rates tend to attract our attention and we often assume that this variability is the

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mechanism regulating populations (e.g., larval survival in amphibians and recruitment in reef fishes). However, across a broad range of organisms with stage-structured populations, there is a strong negative relationship between the variability of a stage specific vital rate and its effect on population growth rate (Pfister 1998). This relationship implies that less variable vital rates, which are often the least studied among organisms with complex life histories, have the greatest potential to regulate populations. Although this is seemingly counterintuitive, evolutionary theory suggests there should be strong selection against variability in the vital rates that bound population size and thereby reduce extinction risk (Vucetich et al. 2000).

Making inaccurate assumptions about the stage at which populations are regulated may not only hinder our understanding of the dynamics of stage-structured populations, but also our ability to conserve them by misdirecting management strategies to focus on the life history stages that are the least likely to reverse population declines (Crouse et al. 1987). With habitat loss and alteration as the leading cause of species extinction, organisms with complex life cycles that require multiple habitat types are at increased risk (Semlitsch 2003). Amphibians are a prime example, with widespread population declines and one-third of species threatened globally (Stuart et al. 2004). Amphibian research and conservation strategies have overwhelmingly focused on aquatic life history stages and the protection of aquatic breeding habitat (Gamble et al. 2006; Rittenhouse and Semlitsch 2007). While aquatic habitat is clearly essential to the viability of these populations, adequate protection of terrestrial habitat may be equally important. A better understanding of the life history stages regulating these populations will aid in the development of demographic models capable of assessing the quantity and quality of terrestrial and aquatic habitat necessary to maintain viable populations, thus allowing the implementation of well-informed management strategies to reverse population declines.

The complex life history of most amphibians consists of an aquatic larval stage followed by terrestrial juvenile and adult stages (Semlitsch and Bodie 2003). Density dependence in the larval stage of amphibians has been clearly demonstrated in numerous laboratory, mesocosm, and pond enclosure experiments (reviewed in Skelly and Kiesecker 2001), with the majority documenting a strong negative relationship between larval density and vital rates including survival, growth rate and size at metamorphosis. The consistency of these results often leads to the assumption that amphibian population regulation occurs in the larval stage. Several studies have documented larval density effects in natural amphibian populations (Newman 1987; Smith 1983; VanBuskirk and Smith 1991); however, few studies have examined the influence of these effects on the growth

rate of the population as a whole. Sensitivity analyses conducted on demographic models for a range of amphibian species demonstrate that pre-metamorphic vital rates have the least effect on population growth rate (Biek et al. 2002; Vonesh and De la Cruz 2002), suggesting that terrestrial stage vital rates may play a greater role in population regulation than aquatic vital rates.

Studies of terrestrial density are rare in amphibians, but experimental manipulations have demonstrated density effects on growth in juvenile *Bufo marinus* (Cohen and Alford 1993), *Rana lessonae* (Altwegg 2003), and *Gastrophryne carolinensis* (Pechmann 1994). However, the functional relationships between terrestrial density and adult vital rates are unknown, making it difficult to incorporate terrestrial density effects into models of amphibian demography. To determine the functional relationships between terrestrial density and survival, growth and reproductive development in two amphibian species, we raised juvenile wood frogs (*Rana sylvatica*) and American toads (*Bufo americanus*) at six densities in 48 terrestrial enclosures. Densities ranged from 1 to 10 metamorphs/m^2. We tracked individual growth and survival over one year. At the end of one year animals were removed from enclosures and we assessed their reproductive development. Using a priori models and a curve-fitting approach, we determined the functional relationships between (1) initial and final density; (2) initial density and final mass. We discuss the implications of our results for understanding and modeling amphibian population dynamics.

Materials and methods

Experimental pens

Pens were located in a field at the edge of a wooded area at the University of Missouri's Research Park (Boone County, MO, USA). Each pen was 1×2 m, constructed of metal sheeting buried 60 cm into the ground with walls extending 60 cm above ground. We dug a hole 30 cm in diameter and 45 cm deep in the center of each pen, filled the hole with leaf litter and covered it with a 60×60 cm plywood coverboard to provide a refuge. We allowed herbaceous vegetation to grow naturally. In addition to the leaf litter under the coverboard, we added approximately 1 kg of dry leaf litter to each pen in the spring and again in late fall. No additional food was added to the pens. All pens were covered with nylon netting (1/4″ mesh) that was secured with plastic conduit. This netting provided additional shade typical of closed canopy wood frog habitat and also excluded predators. During periods when no rain fell for more than ten consecutive days, all pens were watered with the equivalent of 1 cm of rain to reduce the possibility of mortality from

desiccation. We watered pens twice for wood frogs and three times for toads.

Rearing larvae and stocking pens

We collected five wood frog clutches from two ponds in Warren County, Missouri, on 6 March 2004 and 1,000 American toad larvae from a stream in Boone County, Missouri, on 12 May 2005. Wood frog eggs were mixed and kept outdoors in wading pools until larvae were free-swimming. We reared larvae in 1,000 l cattle tanks filled with water, 1 kg dry leaf litter and 1 l of phytoplankton and zooplankton inoculum collected from local natural ponds. Wood frog larvae were counted and added haphazardly to tanks on 26 March 2004 and American toads on 12 May 2005. Wood frogs were raised in 20 tanks at a density of 40 larvae per tank and toads in 10 tanks with 100 larvae per tank. We used different larval densities for each species because natural densities for toads are often much higher than for wood frogs, and also because we wanted to use densities that would yield metamorphs comparable in size to those from local natural populations. In natural populations in Missouri, emerging wood frog metamorphs range in size from 14 to 21 mm and toad metamorphs from 12 to 16 mm (Semlitsch RD, unpublished data). We removed metamorphs from cattle tanks when one or both front limbs emerged, and weighed and measured snout-vent length (SVL) for each animal. Wood frog metamorphs weighed 0.34–0.98 g (mean 0.67; SD 0.12) and were 15–22 mm SVL (mean 20 mm; SD 1.24). American toad metamorphs weighed 0.10–0.32 g (mean 0.18 g; SD 0.04) and were 12–16 mm SVL (mean 13.6; SD 0.87). Metamorphs were individually marked by toe clipping, with no more than three toes clipped per individual. Wood frog and American toad metamorphs were randomly assigned to the 48 terrestrial pens to ensure that there were no initial size differences among treatments (wood frog mass: ANOVA, $F_{(390,5)} = 2.24$; $p = 0.98$; American toad mass: ANOVA, $F_{(390,5)} = 2.24$; $p = 0.70$). Pens were stocked at densities ranging from 1 to 10 animals/m². Estimates of natural terrestrial densities of juvenile wood frogs and American toads are not available in the literature. Annual metamorph production can vary by several orders of magnitude, leading to a broad range of potential natural densities (Berven 1990; Marsh 2001; Semlitsch et al. 1996). Reports of adult wood frog densities range from 0 to 0.75 frogs/m² (Heatwole 1961; Regosin et al. 2003; Roberts and Lewin 1979). Given the high ratio of metamorphs to adults in natural populations [e.g., 20,262 metamorphs to 800 adults (Berven 1990)], terrestrial densities of wood frog metamorphs are expected to be far higher than those of adults. Metamorph densities can be very high near the edges of breeding ponds, e.g., 16–52 animals/m² for southern toads in South Carolina (Beck and Congdon 1999). We stocked the enclosures with a range of densities that is likely at the high end of those found in natural populations because we wanted to determine the potential for terrestrial density dependence to regulate populations, and therefore chose a range of densities that might be characteristic of populations at or near carrying capacity.

Wood frogs occupied the 48 pens from May 2004 to May 2005 and toads from June 2005 to June 2006. We stocked pens with wood frogs from 20 to 26 May 2004 and with toads the following year from 7 to 10 June 2005. Treatments were randomly assigned to pens within each of two blocks of 24 pens. Density treatments of 2, 3, 4, and 7 animals/m² were replicated eight times each; 1 animal/m² was replicated ten times, and 10 animals/m², the highest density was replicated six times. The increased number of replicates of the lower densities was done to improve growth and survival estimates for the treatments in which estimates were necessarily based on fewer animals.

Census protocols

Wood frogs

Censuses were carried out at three-week intervals, with each census being conducted in a 2–4 day period. For wood frogs, we alternated between two types of censuses: (1) a partial census in which three individuals (or two for pens containing only two animals) were haphazardly caught by hand from each pen, identified by individual mark and SVL was measured; (2) a complete census in which pens were thoroughly searched by hand in an attempt to capture, identify and measure all surviving individuals. The first partial census of wood frog pens was carried out on 15–16 June 2004 and the first full census on 5–6 July 2004. A total of three partial censuses and three full censuses were carried out through the summer and early fall. Wood frogs overwintered in the pens and were censused again 27–30 March 2005, coinciding with the breeding season of the natural populations in Missouri. Wood frogs were removed from pens on 12–24 May 2005, with each pen being searched on six different days during this period. Animals were then weighed, their SVL measured, and then euthanized.

American toad

We expected capture probability at each census interval to be lower for toads than for wood frogs because toads are able to burrow, so we increased our search effort by conducting a complete census of all pens at three-week intervals rather than alternating between complete and partial censuses. At each census interval, we identified toads by

individual mark and measured their SVL. We carried out the first census on 28 June 2005 and the last census before overwintering on 29 Sept 2005. After overwintering we conducted one census on 15 April 2006 at the time when natural populations began breeding in Missouri. A final census was conducted on 25 May 2006, during which animals were removed from the pens, identified by mark, their SVL measured, and weighed. We searched the pens an additional five times from 26 to 30 May 2006.

Analysis of survival

All analyses for the two species were run separately because the toad and wood frog experiments were run in separate years with different weather conditions. We used a nonparametric survival analysis to compare survival among the six density treatments for census intervals that occurred before overwintering. Survival analyses were carried out using Minitab 14. Capture probabilities for these intervals were calculated in the program MARK (White and Burnham [1999]) using a Cormack–Jolly–Seber Model to assess the accuracy of our censuses. However, the survival analyses were run using data adjusted for capture probability in the following way. We assumed that capture probability in the one-year census was 100% because pens were searched multiple times in the final one-year census until no additional animals were found. All individuals captured in subsequent censuses were known to be alive in all previous census intervals; they were counted as such and included in survival estimates. This approach may slightly underestimate the number of survivors in each census interval because it does not account for those animals that were missed in a given census and died before being recaptured in a subsequent census. However, this approach resulted in more reasonable survival estimates for individual pens than Jolly–Seber mark recapture estimates, which occasionally yielded population estimates larger than the initial stocking densities.

We estimated the functional relationship between initial stocking density and the density of survivors after one year by fitting five a priori recruitment models to the data using nonlinear regression and ranking the models using Akaike's information criterion (AIC, Akaike [1992]). We considered the individual pens to be the unit of analysis. Analyses were carried out using DataFit 8.1 (Oakdale Engineering, Oakdale, PA, USA). The five recruitment models included: (1) linear function $y = ax + b$; (2) Beverton–Holt $y = (ax)/(1 + bx)$ (Beverton and Holt [1957]); (3) Ricker function $y = e^{-ax}$ (Ricker [1954]); (4) theta-logistic growth $y = x - (x^2/a)^\theta$ (Nelder [1961]); and (5) Shepherd function $y = (ax)/(1 + bx^c)$ (Shepherd [1982]). A positive linear relationship between the initial (x) and final (y) number of individuals/m^2 is indicative of the absence of density

dependence. If density dependence is weak, it is described as a decelerating curve by the Shepherd function when $0 < c < 1$. Compensatory density dependence is represented by a saturating function described by either the Beverton–Holt equation or the Shepherd function (which simplifies to the Beverton–Holt equation when $c = 1$). A hump-shaped relationship between initial and final density represents overcompensatory density dependence, and can be described by the one-parameter Ricker function, which is right-skewed, the two-parameter theta-logistic growth model, or the three-parameter Shepherd function when $c > 1$. Both the theta-logistic and Shepherd models are power functions, which allow a range of curve shapes.

Analysis of growth

We used a repeated measures analysis of variance (ANOVA) to determine differences in growth (SVL) among density treatments over time. Snout-vent lengths were log-transformed to meet the assumption of normality. We analyzed only the data from the equally spaced census intervals prior to overwintering because the repeated measures ANOVA requires equal time intervals. The repeated measures ANOVA does not allow missing values, so we used data only from pens that had at least one capture in each census interval.

For both toads and wood frogs we used a nonlinear regression curve-fitting approach to describe the relationship between initial density and log-transformed body mass after one year. We also evaluated the relationship between density after one year and log-transformed body mass after one year to see if the density resulting from mortality over the course of the year was a better predictor of growth than initial density. We used the pen as the unit of analysis, averaging the mass of individuals in pens containing more than one survivor. We compared the fit of three functions to the data: (1) linear $y = ax + b$; (2) exponential ae^{bx}; and (3) power $y = ax^b$. Models were ranked based on their residual sum of squares.

Assessment of reproductive development

After removal from the pens, wood frogs were euthanized using Tricaine methane sulfonate (MS222), and were dissected to determine sex and stage of reproductive development. Female reproductive development in wood frogs was determined by the degree of convolution of the oviducts (Fig. [1]). Females were categorized as having either: (1) perfectly straight and thin oviducts, as found in metamorphs; (2) oviducts that have begun convolution, but have not yet folded over themselves or widened; (3) convoluted oviducts that fold back over themselves, but have not yet widened, or; (4) convoluted and folded oviducts that have
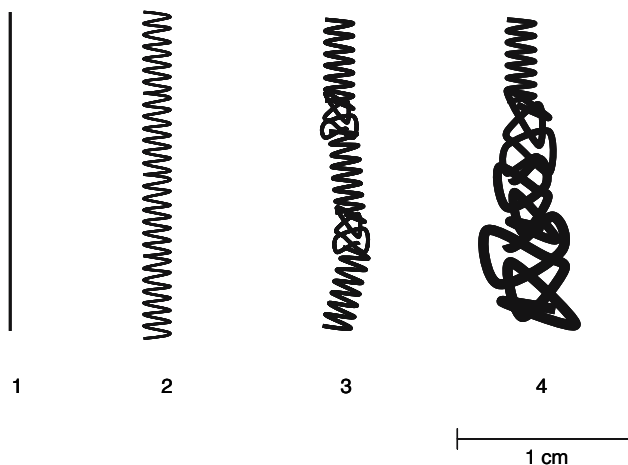
Fig. 1 Categories of oviduct development used to define the degree of reproductive development in female wood frogs (*Rana sylvatica*). Categories are: *1*, immature; *2*, convoluted and narrow; *3*, convoluted and folded over; *4*, highly convoluted and widened

Fig. 2 Average density (number per square meter) of (*top*) American toads (*Bufo americanus*) and (*bottom*) wood frogs (*Rana sylvatica*) surviving in 48 1 × 2 m terrestrial pens stocked at six different initial densities (1, 2, 3, 4, 7 and 10 individuals/m²) from the initiation of the experiment (week 0) through spring until animals were removed from the pens after one year. *Bars* show the standard error of the mean

begun to widen. These categories are similar to those described for *Plethodon cinereus* (Sayler 1966). We compared female wood frog reproductive development scores among treatments using a Kruskal–Wallis test. Male reproductive development was assessed by the presence or absence of nuptial pads, which are secondary sexual characteristics that develop in adult males of most frog and toad species during the breeding season (Di Fiore et al. 2005). American toads were not dissected, but were transferred to larger enclosures to determine their response to density release (results to be reported elsewhere). We compared the proportion of toads in each treatment that reached minimum reproductive size (based on unpublished data from R.D. Semlitsch for natural ponds in Missouri) by the end of the experiment as an indication of reproductive development.

Results

Survival

The differences in survival among density treatments from metamorphosis to overwintering were highly significant for both wood frogs (Wilcoxon; $df = 5$; $\chi^2 = 15.33$; $p = 0.009$; Fig. 2, top) and toads (Wilcoxon; $df = 5$; $\chi^2 = 20.42$; $p = 0.001$; Fig. 2, bottom). Animals in the highest density treatment experienced the greatest mortality with only 28.3% survival in wood frogs (compared to 75% in the lowest density treatment) and even lower survival in toads with only 5.8% of individuals surviving to one year in the highest density treatment (Table 1). At the end of one year, average wood frog densities ranged from 0.75 frogs/m² in
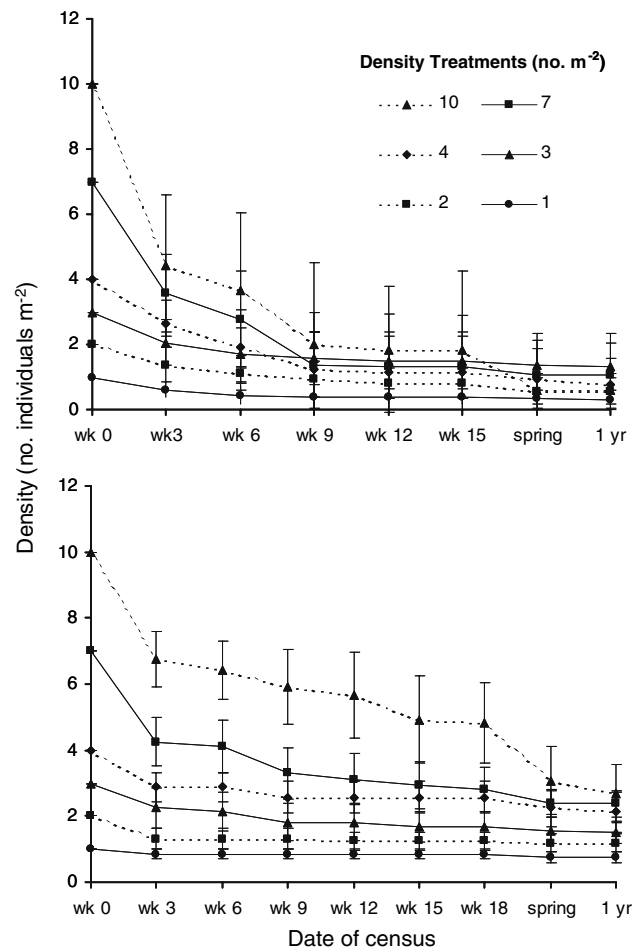
pens initially stocked with 1 frog/m² to 2.7 frogs/m² in pens initially containing 10 frogs/m². Average toad densities after one year were generally lower than in wood frogs (0.31–0.79 toads/m²). However, for toads, pens initially stocked with an intermediate density (4 toads/m²) had the highest average density after one year, rather than pens stocked at higher densities. For wood frogs, the relationship between initial stocking density and density after one year was best described by the theta logistic growth model and was highly significant (Fig. 3a; $y = x - (x^2/5.61)^{0.694}$; $F_{(46,1)} = 57.50$, $p < 0.001$) with good predictive power (r^2 adjusted = 0.55). The theta logistic growth model was also the best descriptor of this relationship for the toads, however, the relationship was not quite significant (Fig. 3b; $y = x - (x^2/2.76)^{0.623}$; $F_{(44,1)} = 3.51$, $p = 0.067$) and had low predictive power (r^2 adjusted = 0.05). Model ranking statistics are shown in Table 2.

Table 1 Percent survival of wood frogs and American toads from metamorphosis to one year

Initial density (animals/m^2)	N (number of pens)	Percent survival of metamorphs to 1 year ($\pm$SE)	
		Wood frogs	American toads
1	10	75.0% ($\pm$8.33)	25.0% ($\pm$8.33)
2	8	59.4% ($\pm$6.6)	28.1% ($\pm$10.0)
3	8	50.0% ($\pm$5.5)	43.8% ($\pm$12.2)
4	8	54.7% ($\pm$7.4)	19.6% ($\pm$9.8)
7	8	34.8% ($\pm$3.7)	17.3% ($\pm$10.2)
10	6	28.3% ($\pm$4.6)	5.8% ($\pm$2.7)

Juveniles were raised in terrestrial enclosures with initial densities ranging from 1–10 animals/m^2

Fig. 3a–b Relationship between initial stocking density and density of survivors after one year ("final density") for **a** toads (*filled squares*) and **b** wood frogs (*filled diamonds*) in terrestrial pens. Sample sizes for the density treatments were ten pens of 1 individual/m^2, eight pens of treatments with 2, 3, 4, and 7 individuals/m^2 and six pens of 10 individuals m^2. The fitted theta-logistic function describing the relationship is shown for both toads ($y = x - (x^2/2.76)^{0.623}$; $r^2 = 0.05$) and wood frogs ($y = x - (x^2/5.61)^{0.694}$; $r^2 = 0.55$)

Table 2 Ranking of models describing the functional relationship between initial density and the density of survivors after one year for wood frogs and American toads

Species	Model	K	AIC$_c$	ΔAIC$_{ci}$	w_i
Wood frog	Theta-logistic growth	2	93.64	0	0.416
	Beverton–Holt	2	93.75	0.11	0.394
	Shepherd	3	95.88	2.24	0.136
	Ricker	1	98.54	4.9	0.036
	Linear	2	99.94	6.3	0.018
American toad	Theta-logistic growth	2	134.93	0	0.393
	Ricker	1	136.09	1.16	0.220
	Beverton–Holt	2	136.60	1.67	0.170
	Shepherd	3	137.06	2.13	0.135
	Linear	2	138.08	3.15	0.081

Model ranking is based on AIC values. K is the number of parameters estimated in each model; AIC_c values are Akaike's information criterion adjusted for small sample size; ΔAIC_{ci} is the difference between the AIC value for a given model and the AIC value of the best approximating model for each data set; w_i values are Akaike weights
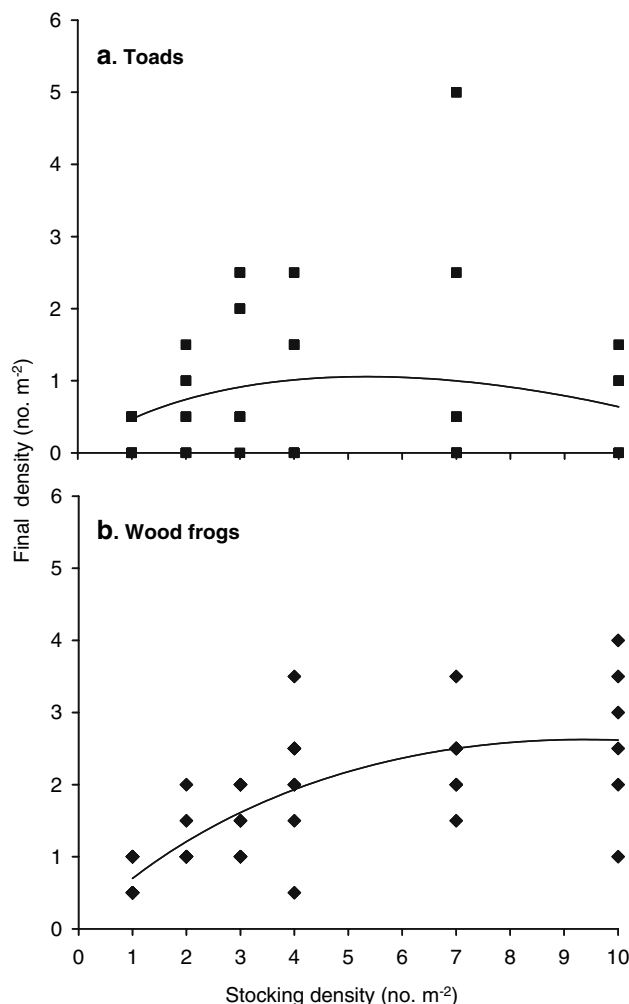
For both wood frogs and toads, the period of greatest mortality across treatments occurred in the first three weeks after the pens were stocked (Fig. 2). For wood frogs this period was followed by consistent but low rates of mortality in five of the density treatments between the first three-week census and the last census before overwintering at 18 weeks (Fig. 2, top). There was no mortality for wood frogs at all in the lowest density treatment during this period. Toads continued to experience high mortality up to the ninth week after metamorphosis followed by lower mortality between week 9 and overwintering (Fig. 2, bottom). During the overwintering period, wood frog survival was lowest (82%) in the highest density treatment, but high (90–97%) within all other treatments. Toads showed a similar overwintering pattern, but with far lower survival in the highest density treatment (32%) and a range of 69–92% survival in the five remaining density treatments. (Table 2)

Mean capture probabilities were high for wood frogs (92–94% among census intervals prior to overwintering and 89–96% among density treatments). Toads were slightly more difficult to detect, with 76–82% probability of capture among census intervals and 71–83% among treatments.

Growth

Growth rates were drastically lower for wood frogs and toads raised at high densities compared to those raised at lower densities (Fig. 4). Differences in growth among density treatments were significant as early as the first three-week census interval and differed significantly among treatments throughout the experiment for both wood frogs (repeated
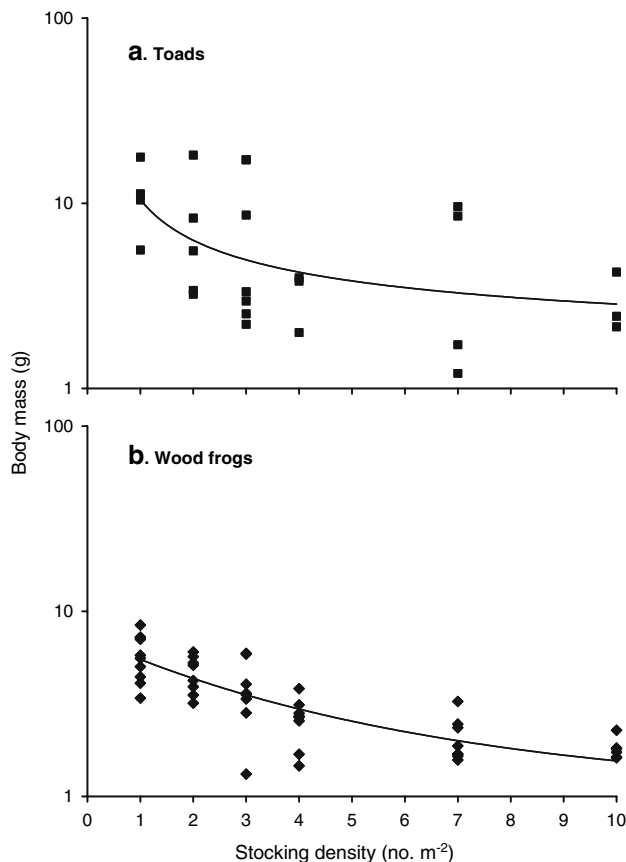
Fig. 4a–b Relationship between initial stocking density of terrestrial enclosures and the body mass of **a** toads (*filled squares*) and **b** wood frogs (*filled diamonds*) after one year. Data points represent the average body mass of surviving animals in each of the 48 terrestrial enclosures. Fitted curves are in the form of a power function for the toads ($\log_{10}$ mass $= 1.02x^{-0.35}$; $r^2 = 0.28$) and an exponential function for the wood frogs ($\log_{10}$ mass $= 0.86e^{-0.15x}$; $r^2 = 0.65$). Note the log scale for body mass
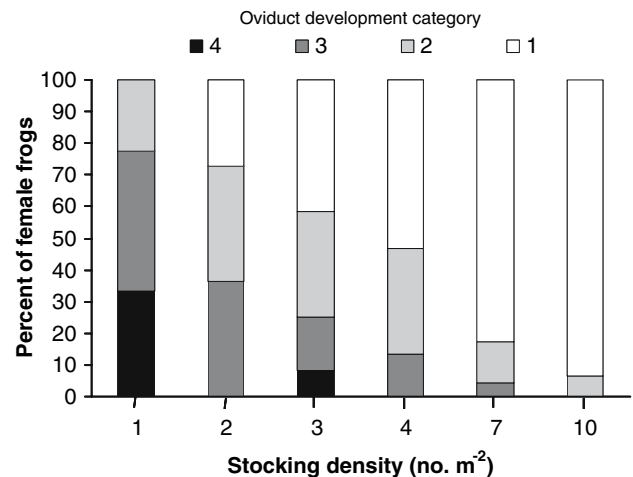

Fig. 5 Oviduct development categories of female wood frogs after one year in terrestrial pens stocked at six initial densities (1, 2, 3, 4, 7, and 10 frogs per square meter). The four oviduct development categories are: *1*, immature (*white bars*); *2*, convoluted and narrow (*light gray bars*); *3*, convoluted and folded over (*dark gray bars*); *4*, highly convoluted and widened (*black bars*). A score of 1 indicates no development, while higher scores indicate that individuals may breed in the following season

measures ANOVA, $F_{(42,5)} = 18.68$; $p < 0.0001$; Fig. 4a) and toads (repeated measures ANOVA, $F_{(12,5)} = 3.67$; $p = 0.030$; Fig. 4b).

Average mass after one year was nearly threefold higher for wood frogs in the lowest density treatment (mean 5.82 g $\pm$ 0.51 SE) compared to those raised at the highest density (mean 1.82 g $\pm$ 0.10 SE), and twice as great for toads in the lowest density (11.24 g $\pm$ 1.94 SE) compared to the highest density treatment (2.95 g $\pm$ 0.66 SE). An exponential function best described the relationship between initial density and log-transformed mass of wood frogs after one year and was highly significant with good predictive power ($y = 0.86e^{-0.15\ x}$; $F_{(46,1)} = 84.95$; $p < 0.0001$; $r^2 = 0.65$; Fig. 5). In toads, this relationship was best described by a power function and was significant with moderate predictive power ($y = 1.02x^{-0.35}$; $F_{(25,1)} = 10.98$; $p < 0.0043$; $r^2 = 0.28$; Fig. 5). Density after one year (i.e., the final density resulting from mortality) was a better predictor of final mass for toads than was initial density ($r^2 = 0.72$ vs. 0.28), but surprisingly, in wood frogs it was not ($r^2 = 0.62$ vs. 0.65).

Reproductive development

Reproductive development was clearly affected by density for both wood frogs and toads. All female wood frogs in the lowest density treatment (1 frog/m^2) had oviducts that exhibited some degree of convolution and over 30% had highly convoluted oviducts that were beginning to widen. Only 1 of 16 female wood frogs (6%) that survived in the highest density treatment (10 frogs/m^2) had any sign of oviduct development. The remaining 15 females (94%) in the highest density treatment had perfectly straight oviducts, as seen in emerging metamorphs. A total of 86 female wood frogs were dissected, with a minimum of nine individuals from each treatment. Reproductive scores were averaged within pens and the difference among treatments was highly significant (Fig. 5; Kruskal–Wallis; $H = 25.71$, $df = 5$, $p < 0.0001$). Male wood frog reproductive development was also affected by density treatments. Only male wood frogs in the lowest three density treatments (1–3 frogs/m^2) developed nuptial pads within one year (Fig. 6).

A total of seven toads, all in the lower three density treatments, reached minimum reproductive size (50 mm SVL; Semlitsch RD, unpublished data) by the end of the experiment. Eighty percent of surviving toads in the lowest density treatment reached reproductive size, compared to
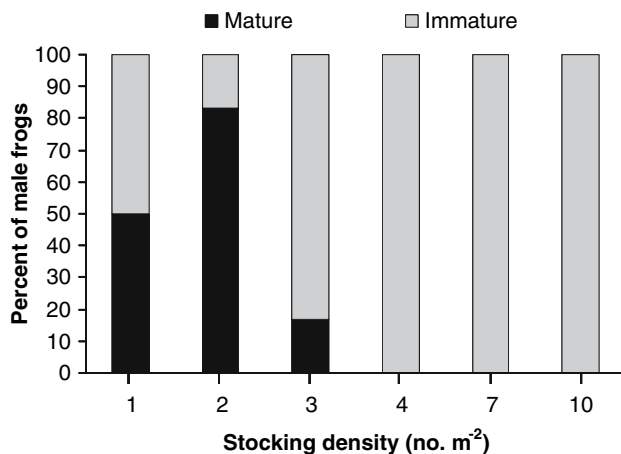
Fig. 6 Percent of male wood frogs that reached reproductive maturity within one year in terrestrial pens stocked at six initial densities (1, 2, 3, 4, 7, 10 per square meter). Mature frogs (*black bars*) exhibited secondary sexual characteristics (nuptial pads) while immature frogs (*gray bars*) did not
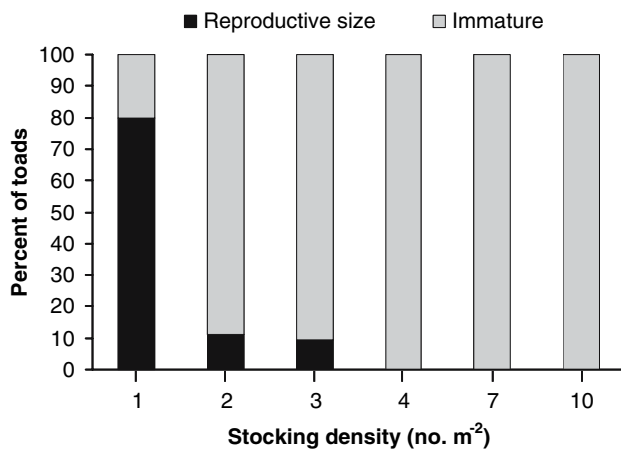

Fig. 7 Percent of American toads (both male and female) that obtained minimum reproductive size (*black portion of bars*) after one year in terrestrial pens stocked at six initial densities (1, 2, 3, 4, 7, 10 per square meter). *Shaded* portions of *bars* represent percentage of toads below minimum reproductive size

11 and 10% in the 2 and 3 toads/m² treatments respectively (Fig. 7). None of the toads in the three highest density treatments reached minimum reproductive size within a year.

Discussion

The effects of terrestrial density on the survival, growth and reproductive development of wood frogs and American toads were unambiguous, with low survival, slow growth and minimal evidence of reproductive development in high density treatments. Overall, toads had far lower survival than wood frogs, which could partly be due to differences in

weather conditions between years. However, an experiment conducted in the same pens in 2002–2003 found similar differences in survival between American toads and another ranid species, *Rana sphenocephala* (James 2005). The fitted theta-logistic growth models describing the relationship between initial density and density of survivors after one year indicate that, across the densities included in our experiment, density dependence is compensatory in wood frogs and strongly overcompensatory in toads. These functions suggest a maximum carrying capacity in the pens of 2.62 frogs/m² for wood frogs and 1.05 toads/m² for American toads. Conditions in the enclosures were favorable, with consistent shade and moisture, and without competition from conspecific adults and other species, suggesting that carrying capacity in the terrestrial environment of natural populations could be much lower than in the enclosures. If that is the case, then density effects would be expected to occur at lower densities, especially in altered and degraded terrestrial habitats.

For both species, the greatest mortality occurred in the first three-week census interval and was primarily density-dependent. However, some mortality may have resulted from density-independent factors as well. Mortality resulting from desiccation is likely greatest during the first weeks following metamorphosis because a low surface-to-volume ratio leads to greater water loss in amphibians (Schmid 1965). Rainfall during the first three weeks of the experiment was relatively high for wood frogs (12.3 cm from 20 May to 15 June 2004; Missouri Historical Agricultural Weather Database, Sanborn Field, MO, USA) and daily maximum temperatures were not extreme (18.3–32.3 °C). Lower rainfall the following year (4.65 cm from 10 to 28 June 2005) and higher maximum temperatures (26.6–33.9 °C) could have contributed to increased early mortality among toads. High mortality soon after metamorphosis has also been documented in larger enclosure studies of *Ambystoma maculatum* and *A. opacum* (Rothermel and Semlitsch 2006) and in natural populations of *A. maculatum*, *A. talpoideum*, and *A. californiense* (Semlitsch 1981; Shoop 1974; Trenham et al. 2000).

Overwintering was also a period of high mortality for both species. Survival during this period was much higher among the lower five density treatments, ranging from 84 to 95% among treatments for wood frogs and from 69 to 92% for toads. The extremely low survival (32%) of toads in the highest density treatment during overwintering is likely the mechanism driving the observed overcompensatory density dependence. Mortality during the overwintering period likely results from an interaction between density-independent physiological limitations and density-dependent body condition and competition for suitable overwintering refugia. Lack of sufficient energy reserves is the most likely cause of density-dependent mortality during this period. In

high density pens, per capita prey availability is lower and animals may not acquire sufficient lipid and glycogen stores, which are critical to survival during overwintering (Pinder et al. 1992). Lipid stores are necessary to fuel metabolism, and in wood frogs glycogen is converted to glucose, which acts as a cryoprotectant (Pinder et al. 1992).

Although mortality reduced the magnitude of the differences in density among the treatments over time, density continued to affect growth throughout the experiment for both wood frogs and toads. Initial density was a good predictor of final mass for both species; however, the relationship had greater predictive power for wood frogs. In toads, final density (i.e., the density resulting from mortality) was a better predictor of final mass than initial density was. This was not the case for wood frogs, suggesting that although mortality may reduce densities, wood frogs may have a low capacity for compensatory growth, leading to the persistence of the effects of initial density. Growth in amphibians is primarily determined by temperature, water, and prey availability (Jorgensen 1992). Temperature and moisture were consistent across treatments, therefore per capita prey availability is the most likely factor leading to reduced growth in the higher density treatments. Food availability has been demonstrated to affect growth rates and reproductive traits in post-metamorphic amphibians (Scott and Fore 1995).

Differences in growth among density treatments had clear consequences for reproductive development in both species. In natural populations, male wood frogs have been documented to reach maturity in one year and females in 1–2 years (Berven 1990; Howard 1980). American toads of both sexes can reach reproductive maturity in one year (personal observation), with the ability to reproduce in the breeding season one year following metamorphosis. In our pens, only animals in low-density treatments reached these reproductive development potentials. None of the animals, male or female, in high-density treatments reached reproductive maturity within a year, and the growth rates exhibited in these treatments suggest that reproductive maturity could be delayed for multiple years.

To understand how the effects of density observed in the pens relate to natural populations, it is important to know the range of densities occurring naturally, but these data are available for very few pond-breeding amphibian species. We are not aware of estimates of natural terrestrial densities of American toads, but some estimates are available for wood frogs. Heatwole (1961) found average densities of wood frogs ranging from 0.13 to 0.75 frogs/m^2 in an upland hardwood swamp in October. The average wood frog densities in our experimental pens by September ranged from 0.85 to 4.8 frogs/m^2 and from 0.75 to 2.7 frogs/m^2 at the end of the study in May. Regosin et al. (2005; 2003) reported average overwintering densities of adult wood frogs ranging from 0–0.063 frogs/m^2 in Massachusetts, and

Roberts and Lewin (1979) found densities of 0.196 wood frogs/m^2 in Alberta. The difference in these estimates of natural wood frog densities, an order of magnitude, suggests that a wide range of terrestrial densities occurs naturally and that the densities used in our experimental enclosures, while at the higher end of what may be found in nature, are not unreasonable.

Terrestrial densities of juveniles are determined by annual metamorph production, rates of dispersal, survival, and habitat selection. As metamorphs move away from the pond, densities are reduced. Juvenile wood frogs migrate primarily in the first four weeks following metamorphosis, after which they settle into summer home ranges, with the majority of individuals settling within 100 m from the pond (Patrick 2007; Patrick et al. 2006). If individuals were distributed evenly within this area, densities might be expected to range from 0 to 0.64 frogs/m^2 in years of high metamorph production (e.g., 20,262 metamorphs as reported in Berven 1990). However, because both wood frog and American toad metamorphs show strong habitat selection (Patrick 2007; Patrick et al. 2006; Rothermel and Semlitsch 2002; Vasconcelos and Calhoun 2004), individuals are likely clumped rather than evenly distributed, leading to high densities of individuals in high quality terrestrial habitat.

The effects of terrestrial density on survival, growth and reproductive development in both wood frogs and toads in our experiments have strong implications for population regulation in the complex life cycles of amphibians. Reduced survival of terrestrial juveniles has been shown to slow population growth rate far more than similar reductions in aquatic larval survival (Biek et al. 2002). Theoretical models have also shown that for the majority of complex life history scenarios, recruitment and adult carrying capacity have a far greater effect on population size than does the carrying capacity of the larval habitat (Halpern et al. 2005). Slow growth rates resulting from high terrestrial densities further reduce population growth rate by increasing the age at first reproduction and increasing the probability of mortality before reaching sexual maturity. Wood frogs rarely live beyond four years in some populations (Berven 1990), and most breeding female American toads are 4–5 years old (Kalb and Zug 1990), so a delay in reproductive maturity substantially increases the probability of mortality before individuals have successfully reproduced and reduces the number of breeding opportunities in a lifetime. Low juvenile growth rates also mean that individuals are smaller at first reproduction, which results in smaller clutch sizes (Berven 1982), further reducing population growth rate.

Although the traditional view of population regulation in amphibians has centered on single-stage regulation at the larval stage, our experimental results, together with several

published field studies of natural populations (Beebee et al. 1996; Berven 1995; Gill 1979; Gittins 1983), suggest that terrestrial-stage or multistage regulation may be common among pond-breeding amphibians, and should be considered in models of amphibian demography. Recent population models have incorporated aquatic density dependence, but have not included terrestrial density effects (Taylor and Scott 1997; Trenham and Shaffer 2005; Vonesh and De la Cruz 2002). Theoretical models have demonstrated that if density dependence is assumed to occur only in the larval stage when it in fact occurs at multiple stages, the resulting population dynamics (e.g., nonoscillatory, oscillatory, or chaotic) could be fundamentally altered (Hellriegel 2000). The way in which density dependence is incorporated into demographic models can also dramatically affect the accuracy of population viability analyses (Henle et al. 2004). Demographic models, including multistage density effects, are crucial in informing management plans for the conservation of organisms with complex life cycles. Accurate models can be used to (1) assess extinction risk; (2) identify the life history stages most likely to respond to management; (3) define the size of reserves necessary to prevent extinction; (4) determine the number of individuals required to establish new populations; (5) set limits on the number of individuals that can be sustainably collected; and (6) determine the number of populations necessary to prevent regional or local extinction (Morris and Doak 2002). However, the accuracy of these predictions depends entirely on the accuracy of the models on which they are based. As areas of suitable terrestrial habitat are reduced and degraded, carrying capacities in the terrestrial environment will likely be reduced, and the effects of terrestrial density may play an even greater role in the dynamics and decline of amphibian populations.

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