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Density regulation in toad populations (*Epidalea calamita, Bufotes viridis*) by differential winter survival of juveniles

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ABSTRACT

The size of amphibian populations varies considerably between years, so that systematic trends in dynamics are difficult to detect. Informed conservation management of presumably declining populations requires the identification of the most sensitive life stage. In temperate-zone anurans there is growing evidence that juveniles hibernating for the first time suffer from substantial winter losses. In two syntopic toads (*Epidalea calamita, Bufotes viridis*) we monitored survival of such juveniles during four consecutive winters in the natural habitat and in four temperature treatments (3°, 5 °C, 10°/15 °C or 20 °C, natural light-dark cycle) in temperature-controlled chambers during winter. Specifically, we tested the hypotheses that (1) winter mortality of juvenile toads which hibernate for the first time in their life is an important component of population dynamics, and that (2) mortality rates differed between the two species. Parameters quantified were size-dependent winter mortality and body condition of pre- and post-hibernating juveniles. Field data provided evidence for the important role of winter mortality of first-hibernators in population dynamics. Choice of hibernacula differed in *E. calamita* between small and medium-sized individuals and also between the two species suggesting distinct mortality risks. The inability of small *E. calamita* to reach frost-proof hibernacula by burrowing, and the exposure of small *B. viridis* to predators are the most probable causes of size-assortative winter mortality. In conclusion, *E. calamita* juveniles may benefit from rising average winter temperatures in the future by decreased risk of freezing to death, whereas predator-caused winter mortality of *B. viridis* juveniles will also depend on the effects of climate warming on predator phenology.

1. Introduction

In nature the size of local amphibian populations fluctuates widely among years, often covering several magnitudes of change (e.g. Meyer et al. (1998) and Pellet et al. (2006)). Long-term fluctuations may camouflage population declines which in some species have led to recent extinction (e.g. Stuart et al. (2004) and Rohr and Raffel (2010)). Causes of population declines are multiple and include habitat destruction, emerging diseases and global climate change (Beebee, 2011). There is growing evidence that climatic change plays a significant role in population declines, either directly via thermal physiology or indirectly by increased susceptibility to pathogens (D'Amen and Bombi, 2009; Catenazzi et al., 2014). For example, increasingly mild winters in the United Kingdom had adverse effects on population viability of *Bufo bufo*, *Epidalea calamita* and *Triturus cristatus* (Reading, 2007; Griffiths et al., 2010; Beebee, 2011), while a montane *Rana luteiventris* population benefited from decreased winter severity (McCaffery and Maxell, 2010). Informed management of declining populations requires a basic understanding of the driving forces of amphibian population dynamics, and the identification of the most sensitive life history stage which determines persistence probability of local populations (Marsh and Trenham, 2001; Hels, 2002; Vonesh and De la Cruz, 2002; Beebee, 2011).

A meta-analysis on the dynamics of amphibian populations shows that abundance fluctuations are larger at the post-metamorphic juvenile than at the adult stage (Marsh, 2001) suggesting particular sensitivity for density regulation at this stage. In *Anaxyrus valliceps*, *A. fowleri* and *Pelobates fuscus*, mortality rate was greatest in juveniles during their first hibernation (Blair, 1953; Clarke, 1977; Schmidt et al., 2012). In the water frog *Pelophylax lessonae,* larval density experienced affected survival during the first hibernation (Altwegg, 2003). In *Lithobathes sylvatica* and *E. calamita,* population size was most sensitive to changes in the survival of juvenile anurans (i.e. 1–2yr old) in an isolated population (Berven, 1990; Di Minin and Griffiths, 2011).

In this study we focus on the density regulation in freeze-

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intolerant populations of the natterjack toad *E. calamita* and the green toad *Bufotes viridis*. These two species have similar ecological niches, share the same habitat, and thus experience the same environmental constraints for hibernation (Sinsch et al., 1999; Sinsch and Leskovar, 2011). Fecundity and number of metamorphs recruited from tadpoles were significantly greater in the green toad *B. viridis*, but *E. calamita* adults outnumbered those of *B. viridis* five- to ten-fold (Sinsch and Keltsch, 2002; Keltsch and Sinsch, 2002). Since first breeders in these species are usually one or two years old (Sinsch et al., 2007,2010), we assume that local density regulation occurs at the juvenile stage. In this study we test the hypotheses that (1) winter mortality of juvenile toads which hibernate for the first time in their life is an important component of population dynamics, and that (2) mortality rates differ between the two species. Specifically, we estimate features of juveniles such as size-specific winter mortality and body condition in their natural habitat during four successive years. Field data are contrasted with those obtained during experimental hibernation in temperature-controlled chambers at four temperatures (3–20 °C). The potential impact of rising average winter temperatures on the survival rate of hibernating juveniles is highlighted.

2. Material and methods

2.1. Study area

Natterjack toads (*Epidalea [Bufo] calamita*) and green toads (*Bufotes viridis*) were studied in a sandy gravel pit area of about 5 km^2 in the Koblenz–Neuwied basin, Rhineland-Palatinate, Germany. The study area was located near the River Rhine at 60–74 m above sea level and included permanent and temporary ponds as well as industrially used areas, cropland and uncultivated land. During the main study period (1998–2002) the two species bred in the shore region of a large pond (surface area: 600–1200 m^2; maximum depth: 1.6 m), and later (2014–2015) in smaller shallow ponds (detailed description in Sinsch et al. (1999, 2015)). The number of breeding females (estimated as annual number of spawn strings 1997–2001) as a proxy of population size ranged between 92–203 in *E. calamita* and 14–49 in *B. viridis* (Sinsch et al., 1999; Sinsch and Keltsch, 2002). Assessment of juvenile abundance was limited to an uncultivated area of ca. 50,000 m^2 with open sandy soil and scattered ruderal vegetation within a 1 km radius of the breeding pond. Local shade temperature was continuously registered by data logger (Tinytalk II, Gemini, one per depth) every two hours at a height of 2 m and 2 cm above ground surface and also in a depth of 20 cm below ground surface in sandy soil (radio tracked adults hibernated in 30–90 cm depth; Sinsch and Leskovar, 2011). Temperature regime during winter (October 1 to March 31 of the following year) was characterized by the daily minima of air temperature at 2 m height and of the underground soil temperature.

2.2. Features of pre- and post-hibernating juveniles in the field

For the purpose of this study we define any post-metamorphic individual which has not yet reached sexual maturity as a juvenile toad. In juvenile *E. calamita*, snout-vent length (SVL) ranged from 7 mm to 43 mm, in *B. viridis* from 9 mm to 44 mm. Individual marking by passive integrated transponders (ID 100 injectable trovan® Transponder, 11 mm length, EURO I.D.) of toadlets was limited to specimens larger than 28 mm (311 *E. calamita*, 397 *B. viridis*). Since the individual ventral pigmentation pattern of small toadlets changes considerably during the first 3 months of life (Meyer and Grosse, 1997), we were unable to distinguish all recaptured juveniles from newly collected, preventing Capture-Mark-Recaptures analysis of census data and subsequent inference of survival rates and detection probabilities (e.g. Schmidt et al. (2012)). Most individuals were first-hibernators, but a few of the larger juveniles (2%) had already experienced a single hibernation (Leskovar and Sinsch, skeletochronological age assessment, unpublished data). As a surrogate measure for the local abundance of juveniles, we assessed the presence of toads in potential daytime shelters (e.g. below stones, planks or in burrows) in standardized census. Each census of shelters lasted 4–6 person-hours, was performed by the same three persons, and always covered the same area of 50,000 m^2. All juveniles were collected, transported to the laboratory, and following species identification snout-vent length (SVL, to the nearest 0.5 mm) and mass (to the nearest mg after emptying the bladder) were recorded for each individual. Within 24 h following census, the juveniles were released again in the shelters at the collecting site. The empirical pre-hibernation abundance count ($C = n$ juveniles per census) integrated the annual variability of fecundity, survival through metamorphosis and survival of toadlets between metamorphosis and the pre-hibernation shelter surveys. Post-hibernation abundance index count reflected the number of juveniles emerging from hibernation and detectable in daytime shelters. Considering the ratio of pre- and post-hibernation abundance a surrogate measure of winter survival rate implies two assumptions (e.g. Schmidt (2003, 2004)): (1) migratory movements to and from the study area did not occur between autumn and spring (closed-population paradigm), and (2) empirical census data C have to be corrected for detection probability d_p to abundance index N. Post-metamorphic dispersal occurred mainly during summer and early autumn (Leskovar and Sinsch, 2005), and juveniles stayed near potential wintering sites during late autumn (Sinsch, unpubl. observ.). While the assumption of a closed-population was probably met for the study period from autumn to early spring, the detection probability was empirically estimated based on the recapture data on the PIT-tagged juveniles for the autumn censuses of 1998–2000 and the spring censuses of 1999–2001. Specifically, we calculated the detection probability for each census day as ratio between the number of tagged juveniles captured and the total number tagged juveniles known to be living (later recaptures). Detection probability d_p for a given survey period was the arithmetic mean of all single estimates obtained and the corresponding standard error. Census data were adjusted for pre- and post-hibernation abundance indices using the formula $N = C/d_p$ (Schmidt, 2003). Pre-hibernation censuses were performed at August 19, September 9 and 24, 1998, at August 28, September 23, 27 and 30, 1999, at August 24, September 11 and 29, October 5, 2000, and at September 12 and 28, October 12, 2001. Corresponding post-hibernation censuses followed at April 20 and 29, May 4, 8, 10 and 17, 1999, at March, 27, April 3, 9, 17 and 23, May 11, 2000, at March 26, May 3, 13, 15 and 29, 2001, and at April 5, 18 and 20, 2002. The irregular intervals between successive surveys were due to short-term cold periods interrupting juvenile activity. After April 21, 2002 the survey area was destroyed because of change in land use. We used the ratio of adjusted post- and pre-hibernation abundance indices as a surrogate measure of survival during winter.

SVL and condition index were recorded as variables potentially related to individual winter survival. The condition index (studentized residuals of the SVL-mass relationship using a multiplicative model $\ln(\text{mass}) = a + b^*\ln(\text{SVL})$, with a = intercept and b = slope; Reading (2007)) is a simple measure describing the state of energy storage of a toad. Raw data used for pre-/post-hibernation comparisons were limited to the censuses after mid-September and before mid-May because the contribution of recent food-uptake to body mass was low and mass mainly reflected the amount of tissue including fat deposits (Leskovar et al., 2004).

All data were tested for deviations from a normal distribution.