Life-history traits in green toad (Bufo viridis) populations: indicators of habitat quality

Ulrich Sinsch, Christoph Leskovar, Anja Drobig, Astrid König, and Wolf-Rüdiger Grosse

Abstract: Five life-history traits (age and size at maturity, longevity, potential reproductive life span, age-dependent growth rate) were investigated in four Bufo viridis Laurenti, 1768 (= Pseudepidalea viridis (Laurenti, 1768)) populations that inhabited localities at similar altitude (60–100 m above sea level) and latitude (50°N–51°N, Germany), but that differed in habitat quality (i.e., human land use within a radius of 1 km around the breeding site). The age of 374 males and of 127 females collected during the breeding period was estimated using skeletochronology on phalange bones. We tested the hypothesis that sex and habitat quality account for detectable amounts of local variation in life-history traits. Significant sexual size dimorphism was present in all populations. Gender-specific variation in size was mainly accounted for by age, but also to a minor extent by habitat quality. In males, age at maturity varied between 1 and 3 years and was the only life-history trait that was significantly related to the intensity of human land use. In contrast, land-use indices co-varied significantly with female longevity (6–15 years) and potential reproductive life span (5–12 years). Our pilot study suggests that, in B. viridis, life-history traits derived from the local age structure may be useful as indicators of habitat quality.

Résumé : Nous avons étudié cinq caractéristiques démographiques (âge et taille à la maturité, longévité, durée potentielle de la vie reproductive, taux de croissance en fonction de l’âge) chez quatre populations de Bufo viridis Laurenti, 1768 (= Pseudepidalea viridis (Laurenti, 1768)) qui habitent des sites d’altitude (60–100 m au-dessus du niveau de la mer) et de latitude (50°N–51°N, Allemagne) semblables, mais de qualité d’habitat différente (c.-à-d. l’utilisation humaine des terres dans un rayon de 1 km autour du site de reproduction). Nous avons estimé par squeletochronologie des phalanges l’âge de 374 mâles et de 127 femelles récoltées durant la période de reproduction. Nous vérifions l’hypothèse selon laquelle le sexe et la qualité de l’habitat expliquent une partie discernable de la variation locale des caractéristiques démographiques. Il y a un dimorphisme sexuel significatif dans toutes les populations. La variation de la taille en fonction du sexe s’explique principalement par l’âge, mais aussi en moindre proportion par la qualité de l’habitat. Chez les mâles, l’âge à la maturité varie entre 1 et 3 ans et c’est le seul caractère démographique qui soit significativement relié à l’importance de l’utilisation anthropique des terres. En revanche, les indices d’utilisation des terres sont en corrélation significative avec la longévité des femelles (6–15 ans) et la durée potentielle de leur vie reproductive (5–12 ans). Notre étude-pilote indique que, chez les B. viridis, les caractéristiques démographiques reliées à la structure en âges locale peuvent servir d’indicateurs commodes de la qualité de l’habitat.

[Traduit par la Rédaction]

Introduction

The life history of an organism consists of its lifetime pattern of growth, development, storage, and reproduction (Begon et al. 1996). It is fixed within certain limits by the genotype of the individual, but the expression of the genotype may vary across different environments, i.e., phenotypic plasticity may be observed (Sorci et al. 1996). Demography is the key to life-history theory because life histories include a set of demographic traits that have evolved in response to environmental factors (Stearns 1992; Heino et al. 1997). Fecundity, reproductive life span, and longevity are such traits that often interact via trade-offs (Stearns 2000). For example, in ectothermic vertebrates, growth decreases greatly after attaining sexual maturity. Reproduction at an early age and small size negatively affect the number, size, and survival of offspring, as well as the survival of parents (Berven 1982; Begon et al. 1996). If annual survivorship is low, the benefits of early reproduction may still outweigh the costs (e.g., Morrison et al. 2004). Thus, lifetime fitness is often more sensitive to changes in the age at maturity than to changes in other traits (Stearns 1992).

Variations in life history among populations of the same species are often explained by differences in habitat quality or climate (Hastings 1997). In amphibians, age at maturity, longevity, potential reproductive life span, and age–size relationships vary consistently in response to latitude and altitude. For example, in Bufo viridis, life-history traits derived from the local age structure may be useful as indicators of habitat quality.
ture among conspecific populations (reviewed in Morrison and Hero 2003; examples include *Bufo bufo* L., 1758) (Hemelaar 1988); *Bufo calamita* Laurenti, 1768 (= *Epidalea calamita* (Laurenti, 1768), according to Frost et al. 2006) (Leskovar et al. 2006); *Bufo hemiophrys* Cope, 1886 (= *Anaxyrus hemiophrys* (Cope, 1886)) (Eaton et al. 2005); *Rana temporaria* L., 1758 (Ryser 1996; Miaud and al. 1999); *Rana sylvatica* LeConte, 1825 (= *Lithobates sylvaticus* (LeConte, 1825)) (Sagar et al. 1998); and *Litoria* spp. (Morrison et al. 2004)). If longevity increases, age and size at maturity also tend to increase (e.g., *Boltoglossa subpalmata* (Boulenger, 1896) (Houck 1982); *Triturus marmoratus* (Latreille, 1800) (Caetano and Castanet 1993); and *Triturus alpestris* Laurenti, 1768 (= *Mesotriton alpestris* (Laurenti, 1768) (Miaud et al. 2000)). An increase in longevity does not necessarily imply an increase in reproductive life span, but as larger females can allocate more energy to the production of offspring than smaller females, lifetime fitness may still increase (Gibbons and McCarthy 1986; Castellano et al. 2004). As reproductive investment differs between males and females, their corresponding age structure may also vary (Charlesworth 1980). The linkage between age at maturity and longevity is pivotal because the evolutionary theory of aging predicts that life span should decrease in response to the amount of mortality caused by extrinsic sources (Stearns 1992; Austad 1997; Kirkwood and Austad 2000).

In this study, we used skeletochronology to determine the age and size structures of four local breeding assemblages of green toads, *Bufo viridis* Laurenti, 1768 (= *Pseudemidella viridis* (Laurenti, 1768), according to Frost et al. 2006). The four study sites were located at similar altitude and latitude, but differed in habitat quality (i.e., human land use within a radius of 1 km around the breeding site). We hypothesize that sex and local land use are significant sources of variation in age and size at maturity, longevity, potential reproductive life span, and age-dependent growth. If so, life-history theory predicts that toads inhabiting habitats with more intense human land use will mature earlier and at smaller size, and have shorter life spans, than those in less disturbed habitats.

Materials and methods

Study areas

Populations of *B. viridis* were studied at four localities in Germany: Urmitz near Koblenz (Rhineland-Palatinate), the botanical garden within the city of Halle, Queis located east of Halle, and Zaschwitz in the Saale floodplain north of Halle (Saxonia-Anhalt). Climate in the Rhine valley (meteorological station: Koblenz-Horchheim, 85 m above sea level, period 1971–2000; Deutscher Wetterdienst, data available at www.dwd.de) is Atlantic with mean summer temperatures of 19.5 °C (July) and winter temperatures of 2.7 °C (January). The mean number of days with temperatures below 0 °C was 44 per year. Total annual precipitation averaged 674 mm. Climate of the Halle region (meteorological station: Halle-Kröllwitz, 96 m above sea level, period: 1961–1990; Müller-Westermier 1996) is continental with mean summer temperatures of 17.8 °C (July) and winter temperatures of 0.0 °C (January). The mean number of days with temperatures below 0 °C was 72 per year. Total annual precipitation averaged 453 mm.

The Urmitz study site (50°21’43”N, 7°35’48”E) is located within the former floodplain of the River Rhine and includes sandy gravel pits, fallow, and arable land. The local toad population was probably established with the initiation of excavation activities about 40 years ago. Toads reproduced in the shallow shore area of a permanent pond within the gravel pit area (Sinsch et al. 1999). The population size (mark–recapture estimate by Lincoln index) was about 100–150 adults (Sinsch and Keltisch 2001).

The Queis study site (Reidepark: 51°29’57”N, 12°10’01”E) is located east of Halle near motorway A14 and includes rain retention basins within arable land and industrial complexes. The present land use, including pond construction, dates back to 1990. Toads reproduced in the shallow shore regions of three permanent, up to 2 m deep, basins that were 50–280 m distant from each other. The population size was about 300–500 adults.

The Halle study site (51°29’03”N, 11°58’16”E) is the botanical garden that was founded in 1698 and expanded in 1787 to its present size of 0.0045 km². The garden is completely walled-in, thus the toad population is isolated from neighbouring populations. Anecdotal reports suggest that the local population has existed for at least 100 years. Toads reproduced in a shallow permanent garden pond. The population size was about 120–150 adults.

The Zaschwitz study site (51°34’39”N, 11°48’28”E) is located in the River Saale floodplain and includes former ox-bows, moist pasture areas, and arable land. Nothing is known about the origin of the local population. Toads reproduced in two permanent eutrophic ponds that were 700 m distant from each other. The population size was about 80–120 adults.

Land use and habitat quality

An a priori assessment of the human impact on the terrestrial habitats within a radius of 1 km around the breeding site (3.141 km²) was based on three dimensionless land-use indices that range from 0 to 1 (Table 1). Most nonreproductive activities of adult toads are thought to occur at this spatial scale (Sinsch 1990; Smith and Green 2005). In case of multiple breeding ponds (Queis, Zaschwitz), circles were drawn around each pond centre and land use quantified within each circle. The arithmetic mean of the two or three local values was used for further analysis at these sites. Land use was quantified using high-resolution topographical maps (1:10000), digital aerial photos, and direct observation during surveys. The dispersal index (DI) is defined as the surface area (%) that does not pose migration barriers to a toad, i.e., the percentage of the area encompassed by geographical barriers. Walls, highly frequented motorways (>500 vehicles per hour), broad rivers, or lakes were considered barriers that impede migration. The numerical value for undisturbed habitats is 1, i.e., no barriers present. The static human impact index (SHI) describes the paved surface area (%) such as roads, human settlements, and industrial complexes. The numerical value for disturbed habitats is 0, i.e., no paved areas present. The dynamic human impact index (DHI) is quantified as the surface area (%) that is subject to frequent alterations (>4

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Table 1. Habitat quality assessment based on three land-use variables.

<table>
<thead>
<tr>
<th>Locality</th>
<th>Dispersal index (DI)</th>
<th>Static human impact index (SHI)</th>
<th>Dynamic human impact index (DHI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urmitz</td>
<td>0.800</td>
<td>0.160</td>
<td>0.490</td>
</tr>
<tr>
<td>Queis</td>
<td>1.000</td>
<td>0.110</td>
<td>0.830</td>
</tr>
<tr>
<td>Halle</td>
<td>0.0014</td>
<td>0.0002</td>
<td>0.000</td>
</tr>
<tr>
<td>Zaschwitz</td>
<td>0.700</td>
<td>0.070</td>
<td>0.450</td>
</tr>
</tbody>
</table>

Note: Details on index estimates are given in the Materials and methods section.

Sampling procedure and skeletochronological age estimation

The assessment of traits in amphibian populations requires a reliable method to estimate age such as skeletochronology (Smirina 1972; Halliday and Verrell 1988). Stainable lines of arrested growth (LAG) detectable in the periosteal bone are ultimately caused by a genetically based, circannual rhythm that under natural conditions becomes synchronized with, and reinforced by, the seasonal cycle (Castanet et al. 1993). Counting the number of LAGs in any type of round bone provides an age estimate with a low margin of error (e.g., Tejedo et al. 1997; Morrison et al. 2004).

A total of 501 adult toads were collected during the reproduction period either at the breeding pond (Urmitz, Halle, Queis) or in drift fences during the breeding migration (Zaschwitz) — (1) Urmitz: 81 males and 13 females in 1998 (17 April – 5 June), 32 males and 29 females in 2000 (27 March – 4 April); (2) Halle: 25 males and 7 females in 1996 (23 March – 2 May), 40 males and 15 females in 1997 (15 March – 27 April); (3) Queis: 104 males and 16 females in 2000 (21 April – 21 May), 63 males and 18 females in 2001 (24 April – 23 May); and (4) Zaschwitz: 29 males and 29 females in 1997 (5 April – 15 May). The large variation in sex ratio is due to females approaching the breeding ponds only for spawning and leaving a few days later, whereas males often stayed for several weeks. Collecting surveys were not exhaustive and samples analysed represent a random sample of the local breeding assemblage. Toads were released again in situ following sex determination, measuring of snout–vent length (SVL, to the nearest millimetre), weighing (to the nearest 0.1 g), and toe-clipping (3rd or 4th toe of the right hind limb). The toes were stored in 70% ethanol at room temperature.

Laboratory protocols followed the standard methods of skeletochronology (Smirina 1972; Kuhn 1994). Samples collected in Halle (1996, 1997), Queis (2001), and Zaschwitz (1997) were embedded in paraffin and cross sections were stained with hematoxylin (for details see Grosse 1999), whereas those obtained in Urmitz (1998, 2000) and Queis (2000) were embedded in Historesin™ (Leica Microsystems, Wetzlar, Germany) and stained with cresylviolet (for details see Sinsch et al. 2001). Differences in embedding and staining procedures do not influence readability of LAGs (U. Sinsch, unpublished data). Diaphysis was cross-sectioned at 10 μm using a Reichert-Jung RM2055 rotation microtome or a LEITZ sliding microtome. Cross sections were examined with a light microscope at magnifications of 200x–400x using an Olympus BX 50 or a Zeiss Amplatz. The number of LAGs was counted in the periosteal bone of those diaphysis sections in which the size of the medular cavity was at its minimum and that of bone was at its maximum. All sections were checked for potential resorption of the innermost LAG by either establishing the presence of the metamorphic line, or if not visible, by comparing the perimeter of the innermost LAG with those of individuals with the metamorphosis line present (e.g., Hemelaar 1988; Kuhn 1994; Olgun et al. 2005).

Life-history traits

The local age structure represented a mixture of cohorts and their respective age-specific mortality and fecundity rates. Because of these limitations, data were not arranged in cohort life tables. Instead, each population was characterized by five sex-specific life-history variables: (1) age at maturity, which is the minimum number of LAGs counted in reproductive individuals; (2) size at maturity, which is the mean SVL of all first breeders with the minimum number of LAGs; (3) observed longevity, which is the maximum number of LAGs counted in reproductive individuals; (4) observed potential reproductive life span, which is the difference between observed longevity and age at maturity; and (5) age–size relationship. As sample size varied considerably among sites, we fitted an exponential regression model to the empirical sex-specific age distributions (equal to survival curves excluding the age class of first breeders) to predict the age distribution at a sample size of 100 individuals. This procedure implies the assumptions that there is no temporal variation in reproduction and survival, and no age bias in capture probabilities. The first assumption is surely violated (interannual variation in Fig. 2), but the effect of temporal variation of empirical age distributions on the precision of the sample-size-adjusted estimate of longevity amounts to ±1 year at most.

Statistical analyses

Estimates of total variability in life-history traits were calculated from the pooled data set for each gender. Medians of age distributions were compared using the Mann–Whitney U test or the Kruskal–Wallis test. Significant differences between the shape of distributions were detected by the two-sided Kolmogorov–Smirnov test. Arithmetic means given with the corresponding standard error (SE) were compared using ANOVA or Student’s t test.

Age–size relationship was estimated using a general linear regression model (GLM). We tested a total of 22 variable transformations to linearize the relationship. The double-reciprocal model provided the best fit (maximum correlation coefficient). The one-factor regression model of age (1/LAG) and size (1/SVL) was complemented by successively introducing sex (categorical variable) and habitat-quality indices (covariates). Variables were added to the model if their corresponding coefficients were significantly different from zero (MANOVA), i.e., they contributed to the amount of variance explained.

The significance of land-use parameters for the life-history
traits age at maturity, longevity, and potential reproductive life span (PRLS) were estimated using the regression model (22 models tested) with the maximum correlation coefficient. Significance level was set at \( \alpha = 0.05 \). All calculations were based on the procedures in STATGRAPHICS Centurion version XV (StatPoint, Inc. 2005).

### Results

Age estimates obtained by scoring the number of LAGs did not seem to be affected by resorption of periosteal bone because the metamorphic line was at least partially visible in 279 of 295 individuals with 3 LAGs or less. In individuals with resorbed metamorphic lines, the distribution of perimeters of the first visible LAG did not deviate from that of first LAGs of individuals with metamorphic lines present (Kolmogorov–Smirnov test, \( p = 0.56 \)).

The minimum age at which sexual maturity was attained was during the 2nd year of life (1 LAG) in both sexes (Figs. 1A, 1B). Maximum life span recorded in reproductive females was 11 LAGs and exceeded that of males by 2 years. The resulting potential reproductive life span was 10 years in females and 8 years in males. Maximum SVL recorded was 86 mm (at 5 LAG) in females and 78 mm (at 3 and 4 LAGs) in males. Longevity was size-independent (Figs. 1A, 1B).

### Age-dependent growth

The double-reciprocal transformation of LAGs and corresponding SVL yielded the best fit for the age–size relationship. Age accounted for 30.5% of the total variation in size (\( F_{[1,499]} = 220.8, \ p < 0.00001 \)). Stepwise introduction of sex as a categorical factor and of three land-use variables (Table 1) as covariates into a GLM resulted in a maximum \( r^2 \) (35.4%) for a model with age, sex, DI, and DHI (\( F_{[4,496]} = \)

### Table 2. GLM model coefficients.

<table>
<thead>
<tr>
<th>Model coefficient</th>
<th>Estimate ± SE</th>
<th>Wilks’ ( \lambda )</th>
<th>( F )</th>
<th>( p )</th>
<th>Contribution (%) to ( r^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>0.0140497±0.0001716</td>
<td>0.7107</td>
<td>201.9</td>
<td>&lt;0.00001</td>
<td>30.50</td>
</tr>
<tr>
<td>Age</td>
<td>0.0051384±0.0003616</td>
<td>0.9445</td>
<td>29.2</td>
<td>&lt;0.00001</td>
<td>3.66</td>
</tr>
<tr>
<td>DHI</td>
<td>-0.0045105±0.00083514</td>
<td>0.9640</td>
<td>18.5</td>
<td>0.00002</td>
<td>0.94</td>
</tr>
<tr>
<td>DI</td>
<td>0.0030593±0.0007104</td>
<td>0.9869</td>
<td>6.6</td>
<td>0.01067</td>
<td>0.39</td>
</tr>
<tr>
<td>Sex</td>
<td>0.0001994±0.00007788</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total ( r^2 ) (adjusted for df values)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>35.40</td>
</tr>
</tbody>
</table>

Note: The model shown here provided the best fit (maximum \( r^2 \)) of the age (1/LAG) to size (1/SVL) relationship, where LAG is lines of arrested growth and SVL is snout–vent length. Numerical range of variables are follows — age: 1–11 LAGs; sex: 1 (male), –1 (female); DI: 0–1; DHI: 0–1.

Fig. 1. (A–D) Age-size relationship in green toads, *Bufo viridis*, from Germany. Scatterplots of 374 males (A) and 127 females (B). Each dot represents an individual toad, with scores per age class slightly jittered to visualize variation. Double-reciprocal regression model are shown for males (C) and females (D), with sex as a categorical factor and habitat-quality indices as covariates. Vertical bars indicate 95% confidence intervals. Model coefficients and statistical significance are summarized in Table 2.
Fig. 2. (A–G) Sex-specific age structure of *B. viridis* populations at four localities and seven breeding periods. (A) Urmitz, 1998: 81 males, 13 females; (B) Urmitz, 2000: 32 males, 29 females; (C) Queis, 2000: 104 males, 16 females; (D) Queis, 2001: 63 males, 18 females; (E) Halle, 1996: 25 males, 7 females; (F) Halle, 1997: 40 males, 15 females; (G) Zaschwitz, 1997: 29 males, 29 females.
Table 3. Key features of life-history traits.

<table>
<thead>
<tr>
<th>Locality</th>
<th>Sex</th>
<th>N</th>
<th>AM (LAGs)</th>
<th>Size at AM (mm; mean ± SE)</th>
<th>Longevity (LAGs)</th>
<th>PRLS (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Observed</td>
<td>Adjusted</td>
<td>Observed</td>
</tr>
<tr>
<td>Urmitz</td>
<td>Male</td>
<td>113</td>
<td>1</td>
<td>50.9±1.7 (N = 14)</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>42</td>
<td>1</td>
<td>48.8±1.8 (N = 4)</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Queis</td>
<td>Male</td>
<td>167</td>
<td>1</td>
<td>55.2±0.9 (N = 10)</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>34</td>
<td>1</td>
<td>55.7±2.1 (N = 3)</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>Halle</td>
<td>Male</td>
<td>65</td>
<td>3</td>
<td>62.5±1.5 (N = 4)</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>22</td>
<td>3</td>
<td>73 (N = 1)</td>
<td>11</td>
<td>15</td>
</tr>
<tr>
<td>Zaschwitz</td>
<td>Male</td>
<td>29</td>
<td>2</td>
<td>64.7±3.3 (N = 2)</td>
<td>9</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>29</td>
<td>3</td>
<td>71.4±2.2 (N = 5)</td>
<td>9</td>
<td>11</td>
</tr>
</tbody>
</table>

Note: AM is age at maturity of the youngest first breeders, size is SVL, and PRLS is the potential reproductive life span.

Table 4. Regression model with the best fit between land-use variables and life-history traits, Pearson’s correlation coefficient, and its significance.

<table>
<thead>
<tr>
<th>Land-use index</th>
<th>AM</th>
<th>Longevity</th>
<th>PRLS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Observed</td>
<td>Adjusted</td>
<td>Observed</td>
</tr>
<tr>
<td>Males:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DI Model</td>
<td>Y = (a + b·X)(^{1/2})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>r</td>
<td>-0.9694</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>p</td>
<td>0.0306</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHI Model</td>
<td>Y = (a + b·X(^{1/2}))^(^{1/2})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>r</td>
<td>-0.9820</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>p</td>
<td>0.0180</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DHI Model</td>
<td>Y = (a + b·X(^{1/2}))^(^{1/2})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>r</td>
<td>-0.9527</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>p</td>
<td>0.0473</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DI Model</td>
<td>Y = (a + b·X)(^{1/2})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>r</td>
<td>-0.9508</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>p</td>
<td>0.0101</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHI Model</td>
<td>Y = (a + b·X(^{1/2}))^(^{1/2})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>r</td>
<td>-0.9999</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>p</td>
<td>0.0101</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DHI Model</td>
<td>Y = (a + b·X(^{1/2}))^(^{1/2})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>r</td>
<td>-0.9314</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>p</td>
<td>0.0286</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: The number of observations is four (localities) in all analysis.

69.5, p << 0.00001; Table 2). Habitat quality accounted for only 4.6% of the total variation in size (Table 2). Age-adjusted size was larger in females (least square mean: 64.4 mm) than in males (62.8 mm; p = 0.0107; Figs. 1C, 1D). In contrast, gender-specific age-adjusted size did not vary significantly among populations (p > 0.05; Figs. 1C, 1D).

Local variation of life-history traits
Age structure differed among populations, as well as between sexes and survey years (Fig. 2). As the number of females collected was low in each year, we restricted the analysis of the year-to-year variation to data on males. At the Urmitz locality, age distribution varied significantly between 1998 and 2000 with respect to median life span (3 LAG vs. 2 LAG; Mann–Whitney U test, p << 0.00001; Figs. 2A, 2B) and to shape of age distribution (Kolmogorov–Smirnov test, p << 0.0001). At the Queis locality, median life span was the same in 2000 and 2001 (2 LAGs; Mann–Whitney U test, p > 0.05; Figs. 2C, 2D), but the shape differed significantly (Kolmogorov–Smirnov test, p << 0.0001). Annual variation of age structure was low in the population at the Halle Botanical Garden, with medians being the same (6 LAGs; Mann–Whitney U test, p > 0.05;
Figs. 2E, 2F) and shape differing slightly (Kolmogorov–Smirnov test, \( p = 0.037 \)).

Local age at maturity showed little sex-specific variation and was 1 LAG at Urmitz and Queis, 3 LAGs at Halle, and 2 LAGs for males and 3 LAGs for females at Zaschwitz (Table 3). First breeders with only 1 LAG were more numerous at Urmitz (12.4% males and 9.5% females) than at Queis (6.0% males and 8.8% females). However, the proportion of individuals breeding at the minimum possible age for maturity was low in all populations. The minimum size at maturity did not differ between the sexes at each locality (Student’s \( t \) test, \( p > 0.05 \); Table 3). However, there were significant differences among the localities (ANOVA; males: \( F_{[3,26]} = 7.7, p = 0.0004 \); females: \( F_{[3,9]} = 30.4, p << 0.0001 \)). Individuals originating from Urmitz and Queis formed one group, which matured at a significantly smaller size than the group composed of individuals from Halle and Zaschwitz (multiple range test with Bonferroni correction, \( p < 0.05 \)).

Sex-specific variation in longevity was low compared with that among the populations (Table 3). Observed longevity was 6 LAGs at Urmitz, 7 LAGs at Queis, 9 LAGs at Zaschwitz, and 9 LAGs for males and 11 LAGs for females at Halle, whereas life-span estimates following sample-size adjustment suggested a longevity of up to 15 LAGs for females at Halle. The resulting PRLS was similar in both sexes, ranging from 5 years at Urmitz to 8 years at Halle based on observed longevity (Table 3).

**Habitat quality and life-history traits**

In males, age at maturity was the only trait that was significantly related to the three land-use variables (Table 4). Small migratory range (low DI) and low static or dynamic human impacts increased the age at maturity. In females, longevity and potential reproductive life span were mainly affected by the size of the migratory range and by the area of the sealed surface (Table 4). Sample-size adjustment for longevity and PRLS improved the sensitivity of the analysis. Low DI and SHI values increased longevity, and consequently PRLS, whereas DHI appeared to be of minor importance.

**Discussion**

Four major categories of habitat quality can be distinguished within the geographical range of a species: (1) unsuitable habitats in which one or more environmental factors impede the establishment of a local population; (2) suitable habitat patches that are unoccupied because of geographic isolation or local extinction; (3) suboptimal habitats that sustain at least temporarily local populations within a source–sink population network; and (4) optimal habitats that sustain long-lasting population with recruitment rates exceeding or balancing loss rates of individuals (e.g., Pulliam 1988). The first two categories are identified in occupancy surveys, and if the specific habitat requirements and the migratory range of the species are known, suitable and unsuitable habitat patches can be reliably distinguished (e.g., Vos and Stumpfel 1995; Bradford et al. 2003). The diagnosis of habitat quality in occupied patches is more complicated because dispersal among neighbouring populations counteracts effectively, and often prevents, local extinction at sink sites (rescue effect); thus, habitat patches with apparently stable local populations vary considerably in quality (Stacey et al. 1997; Bradford et al. 2003). In many species, the local density of populations has been shown to covary with habitat quality (e.g., Franklin et al. 2000; Lin and Batzli 2001). However, measuring local abundances of *B. viridis* is often complicated and time-consuming; therefore, we tested the hypothesis that life-history traits derived from stationary life tables are indicators of habitat quality.

Our study and those of Schnäbele (1987) and Castellano et al. (2004) demonstrate that age and size at maturity, longevity, and PRLS varied widely among populations that inhabit sites of different habitat quality. Variation in life-history traits may reflect neutral genetic variation and (or) phenotypic plasticity in response to environmental factors (Stearns 1992). Nothing is known about genetic variability of Central European *B. viridis* populations, but studies on the widely sympatric *B. calamita* and *B. bufó* with similar Pleistocene recolonization histories indicate a very low degree of genetic polymorphism in populations north of the Alps (Hitchings and Beebee 1996; Beebee and Rowe 2000; Lüscher et al. 2001).

Although overall genetic variability among populations is probably low, we identified gender as a source of variation in life-history patterns. Sex-specific differences in age structure are related to reproductive investment (Charlesworth 1980) and are often observed in amphibians (e.g., Hemelaar 1988; Caetano and Castanet 1993; Miaud et al. 2000). Females tend to mature later than males; to display a greater mean life span, longevity, and PRLS; and to grow larger (Morrison et al. 2004). Though male and female *B. viridis* may mature in their 2nd year of life at a minimum size of 45 mm SVL (this study; Schnäbele 1987), most females delay maturity to their 4th year of life when they have grown to 65–70 mm SVL (this study; Bologna and Giacomina 2006; *B. hemiophrys*, Eaton et al. 2005). Delay of maturity in association with larger body size should allow the production of larger clutches at first reproduction (Hemmer and Kadel 1971; Sinsch and Kelttsch 2001). As age at maturity is related to longevity, delaying maturity does not negatively affect PRLS (e.g., Houck 1982; Miaud et al. 2000; this study). In *B. viridis* populations with delayed sexual maturation, PRLS tended to be greater than in populations with very young first breeders, and sample-size-adjusted estimates suggested even greater differences. Thus, lifetime fecundity of *B. viridis* is probably positively related to age of maturity because older first breeders produce larger clutches and may experience more breeding seasons than young first breeders.

Though gender significantly contributed to the variation in life-history traits among populations, a great proportion of variation remained unexplained. Phenotypic plasticity of demographic traits has been observed in response to altitudinal and latitudinal gradients in many studies on conspecific populations of amphibians (Morrison and Hero 2003; Eaton et al. 2005; Leskovar et al. 2006). Since our study sites have been deliberately chosen at locations of similar altitude and latitude, their potential contribution as sources of variation seem small, although the local climate at Urmitz is, on average, more humid and warmer than at the Halle study sites.
Year-to-year variation in the shape of local age distributions probably reflect stochastic events like unusual drought during summer and intensity of winter.

In contrast, our data on four populations suggest that human impact on the terrestrial surroundings of the breeding ponds, i.e., habitat quality, affects age at maturity in males and longevity and potential reproductive life span in females. As predicted from life-history theory, there was a inverse relationship between the intensity of static and dynamic human impact and age at maturity and longevity, respectively. Research on further B. viridis populations is in progress to increase the currently small sample of study sites and to corroborate the results of this pilot study. In conclusion, we provide the first indication that land use may constitute an important source of variation in life-history traits of B. viridis, and that in turn, age at maturity, longevity, and PRLS are useful indicators of habitat quality.

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