Reproductive ecology of *Bombina variegata*: aspects of life history

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Abstract. Individual reproductive behaviour and survival of *Bombina variegata* was studied in a dynamic habitat with a variety of rain-filled ponds from 1990 to 1992. Most animals reached sexual maturity at the age of two years. Annual adult survival was at least 62%. Individual females, on average, laid between 40 and 70 eggs per "clutch". While about 12% of the breeding females spawned a second time within the season, a similar proportion did not seem to spawn every possible year, probably depending on climatic conditions. The results are consistent with ultimate predictions from life history theory, but the proximate mechanisms of ovulation and spawning in response to environmental conditions remain to be investigated.

Introduction

A fundamental issue in the study of life history is the temporal distribution of reproductive effort (Stearns, 1992). It includes the decisions how often and when to breed. Crucial determinants of these decisions are the mortality during different life stages and the kind and scale of environmental variability. When mortality rate is fluctuating or higher in adult than in juvenile stages, there will be selection for early maturity (Stearns, 1992). But when adult mortality is low, animals can wait for suitable breeding opportunities and spawn several times in their life. This holds for the right timing of breeding within years in seasonal environments, but also for the timing among years and can require total abandonment of reproduction in unfavourable years. Reproduction at older ages combined with bigger size generally provides better energy allocation (Kozłowski, 1992).

Females in most amphibian species are able to reproduce several times in their life and spawn in every possible breeding season (Duellman and Trueb, 1986). Biennial spawning of some anurans is explained by physiological constraints (Bull and Shine, 1979; Silverin and Andrén, 1992), but can also be interpreted as an adaptive reduction

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of reproductive effort (Giesel, 1976). Reproduction can also be partitioned within a season what is widespread among anurans (Perrill and Daniel, 1983; Banks and Beebee, 1986; Denton and Beebee, 1996). Thus, for understanding female reproductive effort, it is essential to know how often females spawn within and over years.

Bombina variegata use various temporary ponds for reproduction (Bauer, 1987). There are reports of long-lived individuals (Seidel, 1992; Płytycz and Bigaj, 1993). So females are likely to spawn several times in their life. In a variable habitat where the existence of spawning sites depends on unpredictable patterns of rainfall, females are also expected to spawn repeatedly during the reproductive season with time of spawning determined by rainfall. The species is therefore considered a suitable organism to study the question of how often and when to breed in a temporally and spatially variable environment.

Study site and methods

The study area is situated north-east of Zürich in a military training area for armoured vehicles. It lies at the border of a former large marsh, 440 m above sea level. One third of the area is covered by a closed mixed forest with little bottom vegetation. The remaining open area is a scarcely vegetated sheep pasture on clay. In this habitat, *Bombina variegata* can be found in an area of about 1.3 km². The study was done between 1990 and 1993 in an area of about 10 ha with the highest density of both ponds and toads. All existing ponds are artificial, created when vehicles compress the soil. A detailed description of the spawning ponds is given in Barandun and Reyer (1997a).

Toads were caught by hand after sunset on 26 nights in 1990, 7 nights in 1991, 14 nights in 1992, and one night in 1993. Only ponds were sampled because few animals could be found on land. Captured toads were kept up to two hours in pails for identification and measurement and thereafter were released into the pond of origin. Both males and females were observed to begin mating behaviour within one hour after being handled. Therefore, the disturbance through capturing is considered minimal. For individual identification, the belly pattern was photographed. All juveniles from 1990 and 1991 received a collective mark in form of two toes clipped in pond- and year-specific combinations. In addition, 23 juveniles were individually identified; they could be recognised by their belly pattern about one month after metamorphosis. Snout-vent-length was measured while stretching the animals between two fingers. Sexually mature males could easily be recognised by their thumb pigmentation. But females could not be distinguished from juveniles unless they carried eggs. The smallest size of a female carrying eggs was 34.8 mm. All individuals larger than this size were classified as females when without thumb pigmentation. The initial classification was checked on every recapture in the same and in subsequent years, but no "sex change" ever became necessary. Therefore, we are confident that the female population was estimated accurately. The number of calling males per pond was recorded approximately twice per week before sampling.

In 1991 and 1992, the presence and number of eggs per pond was counted daily during periods of spawning activity. In 1990, the presence of eggs was recorded only twice per week. Because ponds in this year were not searched for eggs in detail, cases with few eggs per pond may have been overlooked. The total number of new eggs per pond and night is called a "clutch". "Clutch" and egg numbers can be easily determined in *Bombina variegata*, because females lay their eggs in several masses of 1-60 connected eggs and attach them to plants, pieces of wood or other objects in the water (Barandun and Reyer, 1997b). In 1992, 11 ponds were temporarily fenced and egg numbers of 18 individually known enclosed females counted. In six other cases, egg numbers of individual pairs were determined by direct observation of spawning.

Survival and recapture probability were estimated with the maximum-likelihood program SURGE (Lebreton et al., 1992). The program creates ANOVA-like models for survival and recapture probabilities and allows an estimation of the effect of time, age, or sex as potential contributors to the variance. The estimates were based on the annual recaptures of males and females after one and two years. The annual population size was estimated with the Lincoln-index (Seber, 1973) by relating the number of marked and unmarked individuals captured in August to the number of individuals captured and marked earlier in the same year.

Results

Development of juveniles

Juveniles emerged from ponds between June and August each year (Barandun and Reyer, 1997b). The postmetamorphic growth is outlined in fig. 1, starting with the size of 192 juveniles between metamorphosis and first hibernation. Subsequent growth is illustrated by lines representing nine individually identified juveniles of known age and by diamonds showing the size of 24 measured recaptures out of 210 juveniles, marked in 1990. The size of juveniles before first hibernation ranged from less than 15 mm to 28 mm. This broad range may be responsible for the high variability in later size. Most animals reached sexual maturity after two or three winters. Single individuals grew into adult size during the summer following the first winter, but it is unlikely that they took part in reproductive activity in that year. For others, it may take more than three winters to reach adulthood.

Adult survival and population size

In total, 245 females and 354 males were caught and identified individually. On average, each female was captured 2.8 times, each male 4.2 times. The higher capture and recapture rates of males demonstrate that in the sampling area males were present more often and were more active than females. This is especially true for spawning periods when males were up to five times as abundant as females. Males always outnumbered
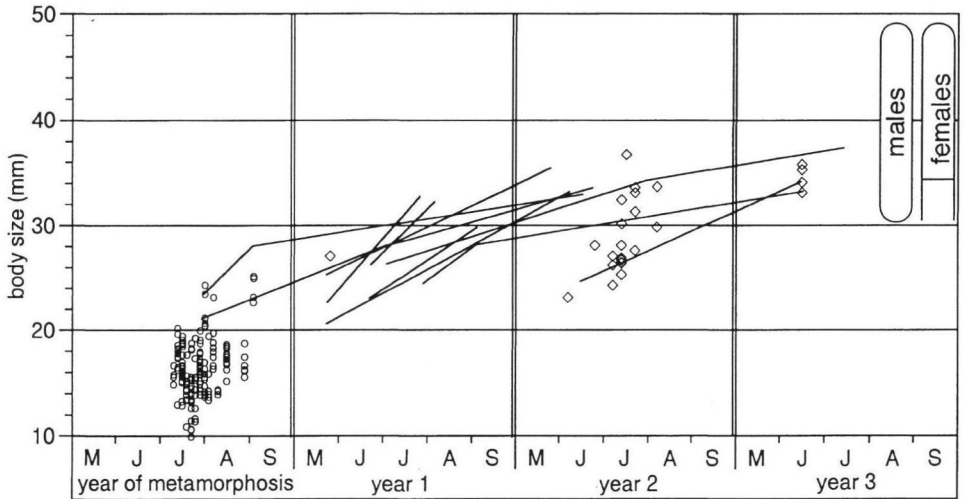
Figure 1. Illustration of postmetamorphic growth from the year of metamorphosis to three years of age. Dots: 192 juveniles measured before first hibernation in 1990, 1991, or 1992; diamonds: 24 collectively toe-clipped juveniles from 1990, recaptured in 1991, 1992, or 1993; lines: growth of nine individually identified juveniles, repeatedly captured between 1990 and 1992. The size ranges of mature males (smallest male: 30.8 mm) and females (smallest female with eggs: 34.8 mm) are shown as boxes. Because identification of small females is unreliable (see Methods), no minimal size is given.

females in spawning ponds. In the forest, which was visited by toads during non-spawning periods, the sex ratio was more balanced. However, females were also found in spawning ponds during periods of reproductive inactivity and in autumn, suggesting that they used ponds for other purposes than spawning. Within a year, 75% of the individually known males and 85% of the females were always found in the same or in an adjacent pond which indicates high site fidelity. The next regularly existing spawning ponds outside the study area were situated more than 500 m away. So, the probability was low that a considerable proportion of the females spawned outside the study area.

Based on recapture data from 1991 and 1992, the average annual survival probability was 58% for mature females and 63% for mature males (fig. 2). The sex independent estimate was 62%. The 95% confidence interval was within 5% for each of the three estimates. However, these figures ignore the recapture probability which varies with the sampling effort and the extent of migration to places outside the sampling area. According to the SURGE estimate, the recapture probability for both sexes was more than 90% in 1992, but only 70-76% in 1991, a year with reduced sampling effort. Thus, actual survival rates must have been higher than 62%.

High adult survival is also indicated by two other observations. First, among 82 toads captured in 1993 (one year after the actual study), 45% were identified as adults from 1990; thus, they had lived at least four years as adults. Second, in 1992 when almost the whole adult population in the study area was sampled, we found only few new adults. In spite of this low recruitment rate, the Lincoln-estimated population sizes of males
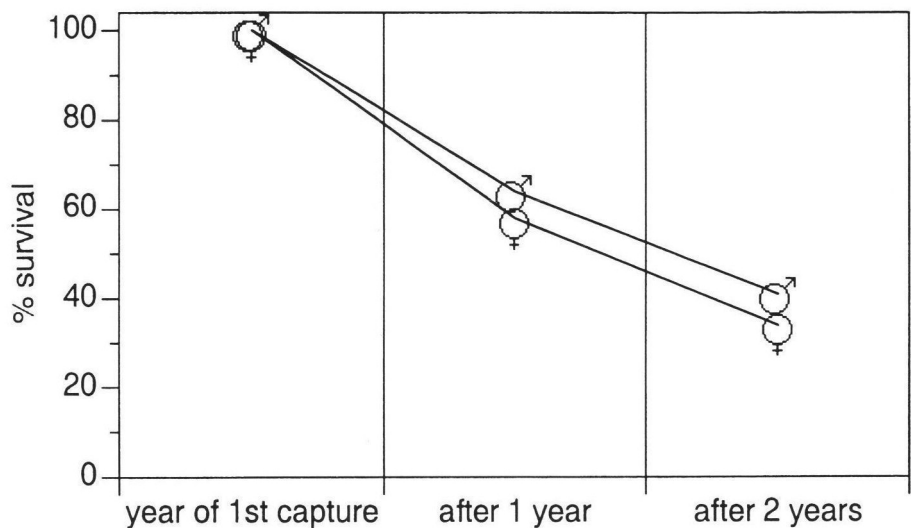
Figure 2. Survival rates of adult toads to one and two years after first capture. Calculations are based on 319 recaptures of 245 individually identified females and 523 recaptures of 354 males.
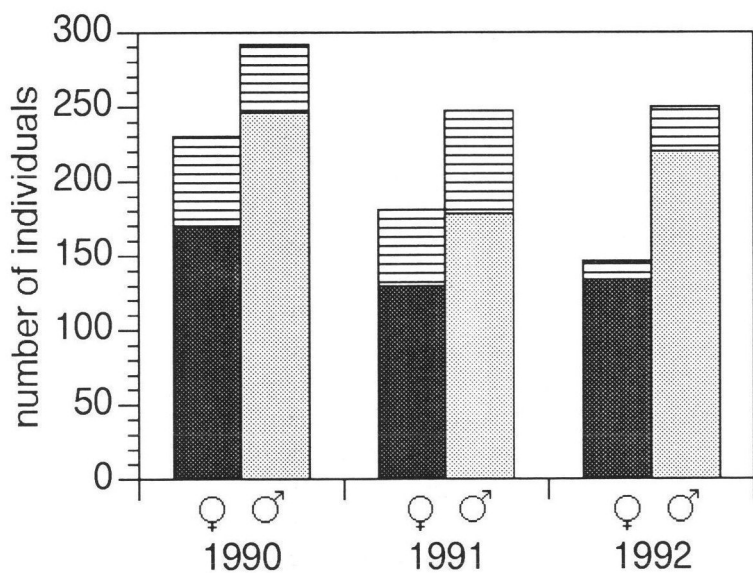

Figure 3. Number of captured individual females (black bars) and males (grey bars) per year and Lincoln estimates of total population size at the end of each year (striped bars).

and females did not decline substantially over the years (fig. 3). This is only possible with high adult survival. Although the above estimates are only rough approximations they allow comparisons among sexes and years. The larger number of males (fig. 3) is consistent with their higher survival rate (fig. 2).

Reproductive output

In our study population, 159 "clutches" were laid during 34 nights with spawning activity in 1991 and 77 "clutches" during 15 nights in 1992. For 72% (1991), respectively 20% (1992), of these "clutches" all eggs were counted. The smallest "clutch" contained 4, the largest 960 eggs (fig. 4); average size was 84 eggs in 1991, 92 in 1992 and 87 pooled over both years. Assuming the same annual averages for the uncounted "clutches" yields a total egg production of 13403 in 1991 and of 7099 in 1992. Given that the number of females differed by less than 20% between the two years (181 in 1991 versus 146 in 1992; fig. 3), this ca. 50% difference in egg production indicates that in 1992 lack of spawning opportunities during droughts did not cause increased spawning during suitable periods. Although there were only half as many spawning nights 1992 as in 1991, the number of eggs laid per spawning night was similar in both years, and so was the average number of ponds used in one night (4.7 and 4.5, respectively; range 0-18).

During the study, 91 females were found in amplexus or dropped eggs when they were caught. They were all considered as spawning individuals. Eleven (12%) of them spawned a second time within a season. This seems to be a good estimate of repeated spawning within the whole population because egg distribution among distant ponds — a potentially confounding factor — apparently occurs neither during the same nor during consecutive nights: 17 out of 21 females that were observed to spawn repeatedly (either within or between years), always used the same pond; the other four females spawned in another pond within 30 m of the previous site.

The number of females taking part in reproduction per year can be estimated indirectly from the total number of eggs and the average size of "clutches", i.e. the appearance of
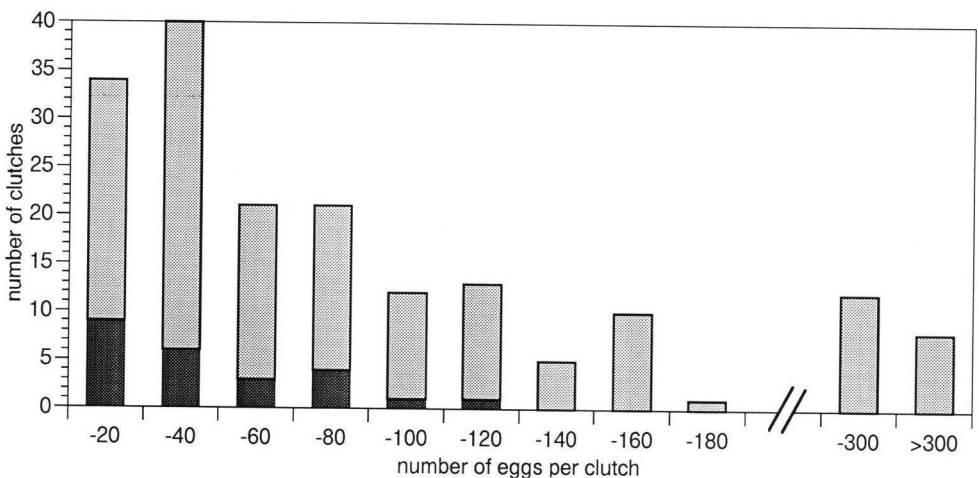

Figure 4. Distribution of completely counted "clutches" (all new eggs per pond and night) in 1991 and 1992 ($n = 177$ "clutches"). Dark bars: egg numbers from individually known females. As no individual laid more than 120 eggs, "clutches" larger than that were assumed to originate from several females.

new eggs per night and pond. For this estimate, the average "clutch" size of 87 calculated above cannot be equalled with eggs per female because — according to literature (Pilniak, 1976; Rafińska, 1991) and our own observations — individual females only exceptionally lay more than 120 eggs per oviposition (cf. dark bars in fig. 4). When "clutches" larger than 120 eggs are attributed to more than one individual, averages of 61 and 65 eggs per female result for 1991 and 1992, respectively. This still may represent an overestimate. In 24 cases, where the egg number per individual female could be counted, the average was only 39. This figure, on the other hand, may be too low, because some of the individuals were disturbed by our sampling and probably interrupted oviposition. Thus the true "clutch" size can be expected to lie somewhere between 40 and 70 eggs per female. Since repeated spawning is rare (see above), this will also represent the total annual egg number for most reproducing females.

Given the total egg numbers of 13403 and 7099, respectively, and assuming the lower end "clutch" size of 40 eggs per female, oviposition occurred 335 times in 1991 and 177 times in 1992. Assuming the upper end "clutch" size of 70 eggs, the corresponding numbers are 191 and 101. Averaged over the two extremes, 263 individual ovipositions occurred in 1991, 139 in 1992. With about 12% of the females spawning a second time within a season, these figures result in 231 females that have spawned in 1991, 122 in 1992. In relation to the estimated total female population in those years (181 and 146, respectively; fig. 3), this implies that virtually all females have spawned in 1991, but only 84% in 1992. The precise numbers will depend on the reliability of our population and "clutch" size estimates as well as on possible differences in repeated spawning between years, which cannot be calculated with only 11 females observed in total. Yet, the above estimates illustrate that some proportion of the females may not spawn every year and that this proportion may fluctuate among years.

Discussion

The majority of the toads reached sexual maturity after two or three winters. But the variability in postmetamorphic growth indicates that many juveniles may need more years to reach adulthood. This is likely to increase the variability of yearly adult recruitment and of reproductive bouts per individual lifetime. Once sexually mature, a considerable proportion of the toads can reach high age as indicated by the annual survival rate of more than 62%. Some adults have become more than 15 years old (Seidel, 1993). For females, this means that they can spawn repeatedly in their life. We did, indeed, find many females spawning repeatedly between years, but repeated spawning within years was rare. The same has been reported for other populations of *Bombina variegata* (Barandun, 1990; Seidel, 1987). This is consistent with bet-hedging theory predicting that — when offspring survival is variable and adult survival is high — there is a large advantage to distribute eggs between years, but only a small advantage to spawn repeatedly within a year (Stearns, 1992).

Two other findings of ours are also consistent with predictions from life history theory. First, under unfavourable circumstances, such as the drought conditions in 1992 (Barandun and Reyer, 1997b), some females may not reproduce at all. Second, compared to most other aquatic spawning anurans (Duellman and Trueb, 1986), annual individual reproductive output is very low in all *Bombina variegata* populations studied so far (Pilniak, 1976; Seidel, 1987; this study). Dissected females have been shown to contain 120 to 300 eggs (Rafińska, 1991). With average "clutch" sizes of 40-70 eggs found in our study, this implies that females may lay only a part of the eggs they produce. On the ultimate level, such conservative reproduction (i.e. small "clutches" and skipping of a whole season) can be explained by the high adult survival rate and life expectancy. On the proximate level, however, the question arises how ovulation as well as retaining and resorbing the eggs is driven by physiological, climatic and other actual environmental conditions. For answering this question, more information about the physiology of egg production and spawning behaviour through lifetime is needed.

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