SPATIAL STRUCTURE AND REGULATION OF A POPULATION OF THE BROWN FROG *RANA MACROCNEMIS* IN GEORGIA

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A spatially structured population of the brown frog *Rana macrocnemis* was studied over seven consecutive years in the valley of a small mountain river. Frogs spawned in numerous temporary pools and puddles situated along the river bed. The overall number of reproductive females varied between 868 and 1146 during the course of study. The population had a 'source-sink' spatial structure: 88% of all froglets metamorphosed in just a few of the sites. Density-dependent mortality of larvae did not significantly affect the overall number of metamorphs. However, the variation in size of the whole population and of one of the two sub-populations was density-dependent. The most important cause of regulation appears to be density-dependent dispersal of juveniles from sources to adjacent sinks. Sinks often had a high number of 'traps' – large well-illuminated pools that frogs prefer for egg deposition but which desiccate during the course of larval development more often than the deep and cold, less attractive breeding sites.

Key words: *Rana macrocnemis*, frog populations, spatial ecology

INTRODUCTION

The concept of spatially heterogeneous populations is an important component of population ecology theory (Hanski, 1996). Two related models are most popular: the 'classic' metapopulation model (Hanski & Gilpin, 1991) and the 'sources and sinks' model (Pulliam, 1988, 1996; Levin, 1989; Howe, Davis & Mosca, 1991). The latter model assumes that a habitat can be subdivided into two parts – sources with a positive reproductive balance, and sinks, that are sustained by migration from sources. Amphibians are favourite subjects for metapopulation studies because their populations can often be subdivided into spatial groupings attached to separate spawning sites. The spatial structure of some amphibian populations can be described in terms of the 'sources-sinks' model (Gill, 1978; Ishchenko, 1979; Sinsch, 1992; Toxopeus *et al.*, 1993; Chubinishvili *et al.*, 1995).

It is not entirely clear how variation in population size relates to spatial structure (Murdoch, 1994). The goal of the present study was to analyse this problem using a population of the Caucasian brown frog (*Rana macrocnemis*). We tried to resolve the following questions: (1) is the variation in size of the entire population and of individual spatial units within this population density-dependent? (2) If the variation in population size is density-dependent, is it regulated during the larval development or after metamorphosis? (3) Are the dynamics of separate spatial units independent or related? (4) How do the dynamics of separate breeding sites affect the dynamics of the whole population?

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MATERIAL AND METHODS

NATURAL HISTORY

Rana macrocnemis belongs to the subgenus *Rana* (*Rana*) which has a wide Holarctic distribution (Dubois, 1993; Green & Borkin, 1993). The reproductive biology of *R. macrocnemis* is similar to that of closely related species (*R. temporaria*, *R. dalmatina*, *R. sylvatica*). Favourable breeding sites are temporary pools. Spawning starts after the melting of snow around breeding sites and is completed in two-three weeks. A female deposits a single egg-clutch containing 200-3500 eggs in a season (Tarkhnishvili, 1993). After the completion of spawning, frogs disperse throughout the terrestrial habitats. They can move at least 5-6 km from a breeding site (Tarkhnishvili, unpublished data). Breeding period, fecundity and terrestrial habits vary in different parts of the species' range (Papanyan, 1961; Kalabekov, 1973; Molov & Ishchenko, 1973; Effendiev & Ishchenko, 1974; Velieva, 1977; Tertishnikov, Logachova & Kutenkov, 1979; Ushakov & Tusnolobova, 1987).

STUDY AREA AND THE DISTRIBUTION OF BREEDING SITES

The study was conducted in the canyon of the river Nedzura (Borjomi district of Georgia), a tributary of the river Mtkvari (Kura). The length of the canyon exceeds 15 km (Fig. 1). Elevation ranges from 900-1000 m (main river bed) to 1200 m (breeding sites situated on mountain slopes). Annual precipitation reaches 1000 mm. The canyon is surrounded by slopes with mixed forest. A few springs feed into the river. Along the river and stream banks, dominant trees are *Alnus barbata*, *Acer campestre*, and *Carpinus caucasica*; on the slopes – *Abies nordmanniana*, *Picea orientalis*,
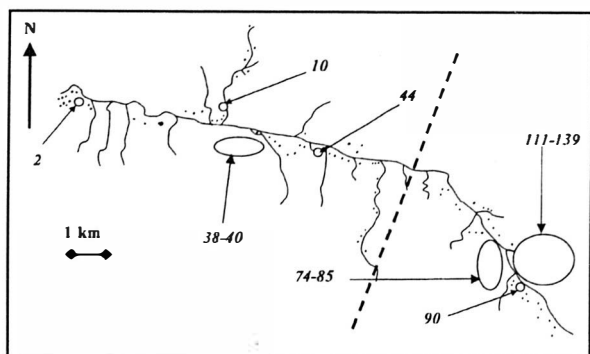
FIG. 1. Map of the study locality. The River Nedzura and its tributaries are shown. Dots indicate separate pools - breeding sites of *R. macrocnemis*. Positions of sites 2, 10, 44, 38-40, 74-85, 90 and 111-139 indicated with corresponding figures. Broken line indicates the border between upper and lower sub-populations.

Carpinus caucasica and *Fagus orientalis*. Breeding sites of *R. macrocnemis* are shared with seven other amphibian species: *Mertensiella caucasica, Triturus vittatus ophryticus, Pelodytes caucasicus, Bufo viridis, B. verrucosissimus, Hyla arborea shelkownikowi* and *Rana ridibunda*. A detailed description of the study area is given elsewhere (Tarkhnishvili, 1993, 1994; Gokhelashvili & Tarkhnishvili, 1994).

Breeding sites of frogs were pools and puddles situated along the banks of the river and streams (Fig. 1). The sizes of individual breeding sites ranged from 2-3 to several thousand litres. During the study period, spawn clumps were recorded at 139 breeding sites. Frogs spawned at 26 of these sites in every year of the study. At other sites, frogs spawned occasionally. 64 breeding sites were in the lower part of the canyon, about 5 km along the lower reaches of the Nedzura (hereafter referred to as the 'lower sub-population'). Seventy-five breeding sites were in the upper reaches of the river (the 'upper sub-population').

The individual breeding sites were rather randomly distributed throughout the study area, although some clearings and pastures had several puddles situated close to each another. Especially dense aggregations of breeding sites are shown in Fig. 1.

ANALYSIS OF THE POPULATION STRUCTURE AND DYNAMICS

Over six consecutive years (1990-1995), each breeding site of *R. macrocnemis* was surveyed every fifth day during the spawning period. Because embryonic development lasted approximately ten days, we were able to count all of the deposited clutches. The total number of clutches was assumed to equal the number of reproductive females. For each individual clutch, the date of deposition and the position of the spawning site were recorded. In 1989, only 13 breeding sites from the upper sub-population were studied.

For 131 clutches collected from all sites in 1989, and for 512 clutches in 1990, the number of eggs per clutch was counted. The methods for the analysis of clutch

size and other reproductive characteristics of the study population (including the correlation between female body size, fecundity and egg size) are described elsewhere (Tarkhnishvili, 1993). The total number of eggs deposited in each pond was then estimated as the product of the number of clutches deposited and the average number of eggs per clutch. The number of eggs in each small (less than 600-800 eggs) or large (more than 2500 eggs) clutch was counted individually.

In 1989-1990, the volume of each spawning site was estimated with the half-ellipsoid volume formula: $v=(\pi abc)/6$, where a and b were maximum length and width of a pool, each divided by 2, and c was its depth. The preferences of frogs for pools of different volumes was estimated with the electivity index of Ivlev (1961): $J=(P_i - P_i{}^*)/(P_i + P_i{}^*)$, where $P_i{}^*$ was the percentage of water bodies of size class i in the environment, and P_i was the percentage of spawn clutches deposited in ponds of this size class. This index was also used to evaluate the level of discrimination between breeding sites of five different types: (1) shallow, well-illuminated pools; (2) pools with a slight current, located in open places; (3) shady, seepage pools in forest, with relatively cold water; (4) cold pools with a slight current, in forest; and (5) brooks.

For the analysis of embryonic and larval mortality (carried out in 1989 and 1990), four sets of data were used: the number of eggs at a breeding site; the number of dead embryos per clutch (for all clutches in ponds containing 1-10 clutches and for ten clutches in ponds containing >10 clutches); the number of tadpoles at a site after the beginning of active feeding (stages 20-25 – Gosner, 1960; generally 3-5 days after hatching was completed); and the number of tadpoles prior to metamorphosis (stages 40-41; generally 2-4 days prior to the beginning of metamorphosis at a site). The latter figure (number of larvae prior to metamorphosis) was used as an index of the number of metamorphs at a site. Moreover, for sites which dried up before completion of metamorphosis, the date of desiccation was recorded. Since metamorphosis was completed in less than one week at most of the breeding sites, individual differences in the developmental rates were not taken into account. The number of active tadpoles was evaluated by three different methods: (1) for the smallest pools (three in 1989 and five in 1990), we simply counted all tadpoles observed at a breeding site; (2) for two shallow pools with the largest water surfaces, the number of tadpoles was counted in each of ten plots of 50 x 50 cm each, the average density per unit pond surface was calculated, and the total number of tadpoles was estimated as a product of the average density and the entire pond surface area; (3) for the other breeding sites (including all pools where metamorphosis was completed) the number of tadpoles was estimated using a mark-recapture technique. We stained tadpoles with neutral red (Guttman & Creasey, 1973), and recaptured them within 4-24 hours of marking (before traces of dye disappeared). A short time interval between marking and

recapture excluded the effect of ontogenetic behavioural changes which could bias the estimated number of tadpoles. The number of tadpoles per site was evaluated using the inverse Petersen index (Caughley, 1977). Recaptures were made until the number of marked individuals in a sample exceeded the figure that ensures a standard error value of less than 10% (Caughley, 1977).

The relative mortality rate for a stage of development was evaluated as the difference between two consecutive estimates, divided by the initial number and multiplied by 100: $Q_x=(N_o-N_t)/N_o \times 100$.

AGE STRUCTURE OF REPRODUCTIVE POPULATION

For some of the adult frogs, we estimated age with standard skeletochronological methods (e.g. Kleinenberg & Smirina, 1969; Castanet & Smirina, 1990). The number of analysed individuals is given in Table 5. Sections (25 μm) of the second phalanx of the fourth toe were stained in Böhmer haematoxylin. Observations on marked individuals indicated that frogs in the study area reach reproductive size (snout-urostyle length > 50 mm) before the second hibernation. Consequently, the first line of arrested growth (LAG, year ring) had a markedly smaller width than the rest of the LAGs and could be easily distinguished. In general, the first LAG was partly or fully resorbed in adults. The LAG representing the last hibernation was not expressed, since we collected samples in early spring (Gokhelashvili & Tarkhnishvili, 1994). The age of individual frogs was calculated as $n+2$ (where n is the number of 'full' LAGs) or (rarely) $n+1$, depending on the degree of resorbtion of the first LAG (Gokhelashvili & Tarkhnishvili, 1994). Age distribution was separately estimated for the two sub-populations and the entire population in 1992, 1993 and 1994. Samples were compared using Kolmogorov-Smirnov lambda-tests and Fisher's angular method (arcsin-transformation of frequencies prior to the pairwise comparison – Sokal & Rohlf, 1995), recommended for comparison of frequencies in samples of small size (Zaitsev, 1984). Multiplying the percentage of separate age cohorts by the total number of clutches in the two sub-populations (see below) and the population as a whole, the total number of reproductive females in a cohort was estimated. The age of first reproduction can vary, but the modal age cohort was assumed to be the greatest age for first reproduction. Mortality rates were estimated as $(N_{i,j}-N_{i+1,j+1})/N_{i,j}$,

where $N_{i,j}$ was the number of frogs of the age class i in year j and $N_{i+1,j+1}$ was the number of the following age class in the next year (where i was greater than or equal to the modal age of the analysed group).

POPULATION REGULATION HYPOTHESES

To detect temporal trends in the dynamics of individual breeding sites, sub-populations and the entire population, the approach of Varley & Gradwell was applied (Southwood, 1996). Prior to the analysis, the number of clutches was *ln*-transformed. The k-value, indicating the change in number between two consecutive years, was estimated as: $k=ln\ N_t - ln\ N_{t+1}$. For individual breeding sites, the two sub-populations and the whole population, the measure of density dependence, b, was estimated as the coefficient of regression of k on $ln\ N_t$. If $0.5<b<1.5$, the dynamics of a spatial grouping were assumed to be density dependent; if $b<0.5$, dynamics were assumed to be density independent; and if $b>1.5$, the dynamics were assumed to result from an overcompensating effect (Southwood, 1996). To estimate the statistical significance of the values of b obtained, the correlation coefficient between k and $ln\ N_t$ was calculated.

RESULTS

SPATIAL STRUCTURE

The number of breeding sites varied from 73 - 97 between years. The number of sites in the lower sub-population declined from 49 in 1991 to 29 in 1993. The number of sites in the upper sub-population increased from 43 in 1990 to 62 in 1995.

On average, 11.5±0.6 clutches per year were deposited at each site (range: 1-113; CV=123 %; N=560; range of means: 11.32-13.44 for different years; Fig.2). The distribution of clutches throughout breeding sites was strongly aggregated. Some 58% of clutches were deposited at 3.9% of breeding sites, each of which contained 20 or more clutches (Fig. 1). Frogs preferred well-illuminated, warm, shallow pools with a volume of more than 160 l (type 1), and avoided spawning in cold, slowly running waters, and in pools with a volume of less than 20 l (Table 1).

DYNAMICS OF REPRODUCTIVE FEMALES

The numbers of clutches found at each breeding site and at the two sub-populations are shown in Table 2

TABLE 1. Electivity of spawning sites of different type and volume. Data for 1989 and 1990 are pooled. Types of spawning site: (1) shallow, well-illuminated pools; (2) pools with a slight current, located in open places; (3) shady seepage pools in forest, with relatively cold water; (4) cold pools with a slight current, in forest; and (5) brooks.

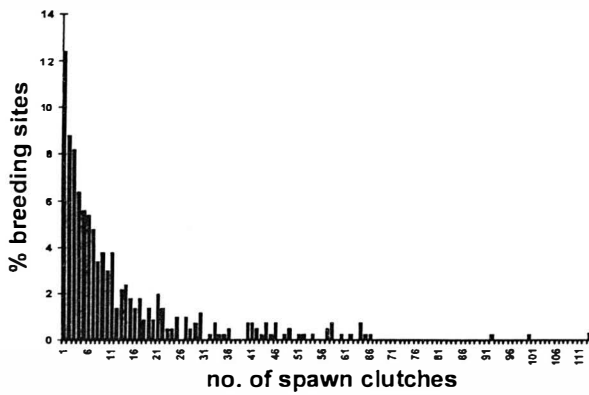| Type of spawning site	1	2	3	4	5
Electivity index	+0.12	+0.06	-0.09	-0.10	-0.71
Volume of spawning site	<20	20-160	160-1280	>1280	
Electivity index	-0.31	+0.05	+0.26	+0.28	

FIG. 2. The distribution of breeding sites according to the number of deposited spawn clutches, using combined data for 1989-1995.
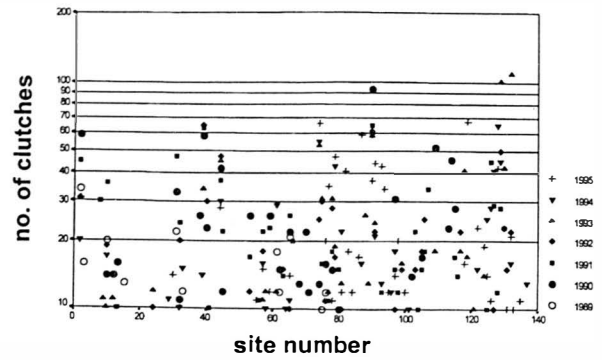

FIG. 3. Dynamics of clutch numbers at 139 individual breeding sites. Sites 1-64 belong to the lower sub-population, sites 65-139, to the upper sub-population. Numbers <10 not shown.

TABLE 2. Dynamics of clutch numbers for the entire population (EP), and lower (LSP) and upper (USP) sub-populations during the study period.

	1990	1991	1992	1993	1994	1995
LSP	479	539	352	264	254	219
USP	546	560	516	798	645	927
EP	1025	1099	868	1062	899	1146

and Fig. 3. The total number of clutches across all sites varied between 868 and 1146. The average number of reproductive females was 1017, with CV=11%. During the course of the study, the number of clutches declined within the lower sub-population, but increased for the upper sub-population.

MORTALITY FROM EGG TO METAMORPHOSIS

Eggs and larvae did not survive in 43 pools in 1989 (78% of breeding sites) and in 79 pools in 1990 (89%) (Fig. 1). From 1991-1995, metamorphosis never took place at more than 20 breeding sites. Desiccation was the commonest cause of mortality. The total number of clutches at sites that desiccated before metamorphosis

was 118 (43.1%) in 1989 and 796 (77%) in 1990. In other breeding sites, the average survival rate from egg to metamorphosis was 5.99% (0.03-23.3%, SE=5.8). The total number of surviving offspring was 4.45% from deposited eggs in 1989 and 3.01% in 1990 (Table 3). The distribution of clutches did not differ between desiccated and successful breeding sites: $\chi^2=10.2$ (1989), $\chi^2=4.0$ (1990) ($N=11$, $P>0.05$). The distribution of metamorphs throughout individual sites and plots was even more aggregated than the distribution of eggs. In 1989, 82% of the new generation emerged from two sites; in 1990, 70% metamorphosed from a single site (Table 4).

In 1989, the initial density (number of eggs per unit of water volume) was significantly correlated with mortality rate between hatching and active feeding for 32 breeding sites: $r=0.41$, $P<0.05$. For ten sites where metamorphosis was completed, the mortality rate between the beginning of active feeding and metamorphosis was related to the density of newly hatched tadpoles: $r=0.68$, $P<0.05$. The regression was curvilinear and the correlation between log-transformed values reached 0.98 ($P<0.01$). However, the density-dependent mortality was undercompensating: the number of offspring was positively related to the

TABLE 3. Output of the population, in terms of the number of clutches deposited, egg and larval survival, and offspring number. Combined data for 1989 and 1990. Nc, the total number of clutches deposited; Ne, the total number of eggs; Pe, survival of eggs, %; Pl, survival of larvae before active feeding, %; Pt, survival of tadpoles before metamorphosis, %; Pem, survival from egg to metamorphosis, %; Nm, the number of metamorphosing tadpoles. A, combined data for all breeding sites; B, sites which did not desiccate before metamorphosis; C, sources. Whole population (1990), studied part of the population (1989).

		Nc	Ne	Pe	Pl	Pt	Pem	Nm
A	1989	274	401,136	61.2	31.0	-	4.45	17,845
	1990	1025	1,518,168	?	?	?	3.01	44,600
B	1989	156	242,619	88.4	14.3	58.3	7.36	17,845
	1990	241	315,368	?	?	?	14.14	44,600
C	1989	74	152,321	87.4	15.6	78.3	10.67	16,247
	1990	150	207,467	?	?	?	18.75	38,903

TABLE 4. Reproductive output of individual breeding sites and sub-populations. Only those sites where some larvae reached metamorphosis are shown. #BS, number of a breeding site (code); Ncl, number of clutches deposited at a site; Nm, number of individuals that reached metamorphosis; Qem, percentage survival from egg to metamorphosis; %C, percentage input of a plot to a new cohort. LSP, lower sub-population; USP, upper sub-population. T, total figures for respective sub-populations.

1989

	LSP												TLSP	USP
#BS	2	10	14	15	17	18	31	32	44	50	53	59	**1-64**	74
Ncl	34	20	4	13	3	5	22	4	7	6	4	5	**273**	7
Nm	8964	4109	291	1174	105	10	687	2	~200	6	364	119	**17831**	14
Qem	12.7	8.5	4.3	4.3	2.3	0.12	3.1	0.08	19.2	0.14	6.1	2.4	**3.7**	0.2
%C	50.3	23.0	1.6	6.6	0.5	~0	3.9	~0	11.2	~0	2.0	0.1	**~100**	~0

1990

	LSP			TLSP	USP							TUSP
#BS	44	45	59	**1-64**	78	80	90	91	92	93	113	**65-139**
Ncl	42	12	26	**413**	15	10	93	4	7	9	23	**612**
Nm	~5000	102	1800	**6902**	3000	500	30903	1000	1000	250	1000	**37653**
Qem	8.0	0.92	6.22	**1.1**	14.2	4.4	23.3	17.5	10.0	1.9	3.0	**4.2**
%C	11.2	0.2	4.0	**15.5**	6.7	1.1	69.4	2.2	2.2	0.5	2.2	**84.5**

number of deposited eggs. The correlation of log-transformed data, combined for 1989 and 1990, reached 0.99; the regression is described by the allometric equation $y=0.00039x^{1.52}$, where y is the number of tadpoles prior to metamorphosis and x is the number of deposited eggs (Fig.4).

SPATIAL DISTRIBUTION OF METAMORPHS AND ADULT FROGS

The number of adult females in each of the two sub-populations was dependent on the number of metamorphs in the same sub-population 2-3 years earlier. In 1989, the majority of surviving offspring came from three pools in the lower sub-population (Table 4).
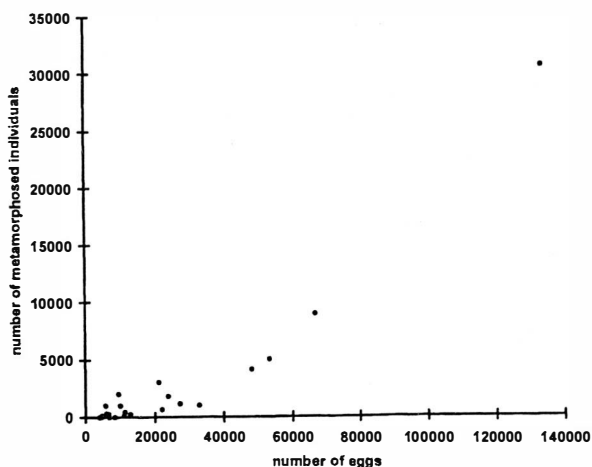

FIG. 4. The relationship between the number of eggs deposited at a site and the number of tadpoles reaching metamorphosis. Only those breeding sites where metamorphosis was observed are included.

In 1991, the number of clutches in this sub-population increased 1.13 times, whereas those in the upper sub-population increased 1.02 times (in comparison with the previous year). In 1990 the majority of surviving offspring came from a single pond, no. 90 (upper sub-population; Table 3). Between 1990 and 1993, the number of clutches in the upper sub-population increased 1.46 times, whereas those in the lower sub-population decreased by almost a half (Table 2, Fig. 3).

AGE DISTRIBUTION

The age structure of reproductive frogs is shown in Table 5. The age of the vast majority of individuals did not exceed five years. Inter-sexual differences were insignificant (Kolmogorov lambda <1.36, $P>0.05$ for each year). The age distributions of frogs from the lower and upper sub-populations did not differ significantly, according to Kolmogorov's lambda, but the differences were significant according to Fisher's angular test. In 1993, the proportion of 3-year-old frogs (the cohort originating in 1990) was significantly higher in the upper sub-population: $F = 6.20$, $P<0.05$, df = 52. Mortality rates differed between cohorts and between the two sub-populations. Survival rates in cohorts that originated in 1989 and 1990 were, respectively, 23 and 27% per year during the first three years of life; during the following year, mortality rates reached 35% for the cohort originating in 1990 (upper sub-population) and 43% for the cohort originating in 1989 (lower sub-population).

TEMPORAL TRENDS IN THE DYNAMICS

The factor b for the whole population reached 1.93 ($P<0.05$). The corresponding values were 0.15 for the

TABLE 5. Age distribution of reproductive frogs in 1992-1994. Percentages of specimens in different age classes and the mean ages of reproductive animals are shown. N, sample size; figures for modal age classes are shown in bold type; m, males; f, females; Nf, the absolute numbers of females of different age classes, calculated as the products of the percentages and the total numbers of clutches deposited.

sex	year	N	Age classes (years)						Mean	SD
			2	3	4	5	6	7		
(1) Whole population										
m	1992	50	**42**	**42**	10	6			2.78	0.85
m	1993	42	26	**48**	14	10	2		3.13	0.91
m	1994	83	11	29	**41**	19			3.68	0.90
f	1992	18	22	**39**	28	11			3.39	0.98
f	1993	12	8	25	**42**	8	8	8	4.03	1.10
f	1994	14	14	29	**36**	14	7		3.71	1.14
Nf	1992		191	**339**	243	95				
Nf	1993		86	262	**442**	86	86	86		
Nf	1994		126	261	**323**	126	63			
(2) Lower sub-population										
m+f	1992	32	**53**	38	9				2.56	0.66
m+f	1993	34	**32**	26	29	9	4		3.27	1.12
m+f	1994	31	19	**38**	29	14			3.00	1.02
Nf	1992		**186**	134	32					
Nf	1993		**83**	69	77	24	11			
Nf	1994		48	**96**	74	36				
(3) Upper sub-population										
m+f	1992	26	37	**41**	11	11			2.96	0.96
m+f	1993	20		**60**		20	10	10	4.10	1.45
m+f	1994	66	8	21	**48**	21	2		3.88	0.90
Nf	1992		190	**212**	57	57				
Nf	1993			**478**		160	80	80		
Nf	1994		52	135	**310**	135	13			

lower sub-population was 0.15, and 0.81for the upper sub-population (both $P<0.05$). Thus, the variation in size of the lower sub-population was density-independent; of the upper sub-population density-dependent, and for the entire locality density-dependent with over-compensation. For individual breeding sites, no significant density-dependence was observed.

DISCUSSION

The study population has a sources-sinks type of spatial organization (Pulliam, 1988, 1996). Every year, a few breeding sites (sources) provided the majority of metamorphs, while at the other breeding sites all or almost all larvae died before metamorphosis. Successfully breeding females represented 15-27% of the population. The spatial position of sources varied from year to year. The dynamics were density-dependent and the range of variation was lower than in many other amphibian populations (Gill, 1978; Ishchenko & Ledentsov, 1987; Berven & Grudzien, 1990; Sinsch &

Seidel, 1995). Could the sources-sinks type of spatial structure be responsible for the observed stability? The key to this question is in the analysis of the dynamics of individual spatial units.

Density-dependent population dynamics could be connected with either density-dependent mortality during the larval period (Shoop, 1974; Tarkhnishvili & Pyastolova, 1985; Severtsov & Surova, 1989; see Wilbur, 1996 for review), or with density-dependent mortality, immigration and individual growth rates in the terrestrial stages (Ishchenko, 1983, 1989; Lyapkov,1995). Mortality of larvae was density-dependent at sites which did not desiccate. However, most of the breeding sites desiccated. This reduced the importance of density-dependence for mortality, as in many other amphibian populations (Semlitsch, 1987; Cooke, 1975; Kuzmin & Godina, 1986; Albers & Prouty, 1987). Moreover, a strong positive correlation between the numbers of eggs and metamorphs was recorded: this indicated that the variation in larval

mortality between breeding sites was not regulating numbers. Consequently, small variations in the population size appear to be connected with demographic events that occured after metamorphosis.

A plausible explanation is that dispersal prevented both over-population of sources and extinction of sinks. The number of offspring at an individual site influences the number of reproductive females in all neighbouring sites a few years later. For instance, the total number of frogs in the upper sub-population increased significantly (by 46%) three years after a year when a large number of juveniles emerged from breeding site no. 90. This population growth does not apply to site no. 90 itself, i.e. most of the offspring from 1990 did not return for breeding to their pond of origin. A similar situation was observed for the lower sub-population, whose size increased by 12% after two years, since the most productive breeding sites were nos. 2, 10 and 44. Analysis of the age structure of reproductive frogs supports a relationship between the number of offspring that developed in sources and the number of adults that appeared in the same sub-population over following years.

Sites 111-139, located within a large, well-illuminated pasture (Fig. 1), were the most important recipients of the cohort which originated from site no. 90 and a few adjacent pools. In 1993, the number of reproductive females at these sites increased by 237 in comparison with the previous year. The number of females in another, adjacent group of sites (74-85, which were located in forest) increased by just 62 (Fig. 3). Collectively, these breeding sites ensured the survival of 3.3% of the deposited eggs, a much higher rate than sites 111-139. Was the negative association between survival of larvae and immigration rates accidental? Sites 111-139 are shallow, warm, well-illuminated pools, which frogs prefer for spawning. Most of these pools desiccated soon after spawning. Thus, preferences of reproductive females did not ensure an increase in reproductive fitness. Other observations supported the absence of association between preferred and effective sites. For example, at site no. 2 spawning took place until 1995, though no larvae survived there after 1989. The number of clutches at sites 38-40 remained high between 1992 and 1995, though no successful metamorphosis was observed there until 1995. All these sites are shallow and warm pools. It is hard to explain the preference for non-optimal sites. Possibly these sites provide 'comfortable' conditions for breeding adults that simply prefer to stay in relatively warm water (or perhaps survive better if water is not so cold), and this factor has a stronger impact than the survival of eggs and larvae on the selection of breeding sites.

Ray & Hastings (1996) showed that, for insects, density-dependence in metapopulations is more often detected for mobile than for immobile stages of the life cycle. Perhaps this conclusion also applies to amphibians. Simulation models support the point that local migrations can increase the density dependence of a metapopulation (Hanski, Foley & Hassell, 1996; Howe *et al.*, 1991). This process is accelerated if sinks attract reproductive specimens more than sources (such types of sinks Pulliam (1996) called 'traps'). In the study area, presence of traps determines the main directions of dispersal (mainly from sources to sinks) and strengthens density-dependence in overall population dynamics.

An additional mechanism of regulation appears to be density-dependent individual growth and mortality. In 1990, the source was in the upper sub-population and twice as many metamorphs emerged than in 1989, when the source was in the lower sub-population. Consequently, frogs that metamorphosed in 1989 from the lower sub-population began to reproduce (on average) after two hibernations, whereas frogs that metamorphosed in 1990 began to reproduce after three hibernations (Table 5). A similar postponement of maturation in cohorts with a high initial density was recorded for *Rana arvalis* (Ishchenko, 1989; Ishchenko & Ledentsov, 1987). Late-maturating cohorts had lower annual mortality rates than early-maturating cohorts, as in other brown frogs (Berven, 1988; Ishchenko & Ledentsov, 1987).

It is notable that the number of frogs was more stable and well-regulated for the entire population than for the two separate sub-populations. The decline of the lower sub-population was caused by anthropogenic influences in the 1990s, when some breeding sites were destroyed. The reasons for a simultaneous increase in the upper sub-population remain unclear. The regulatory mechanisms described above do not provide a plausible explanation because the dispersal rates between lower and upper sub-populations appear to be low. Because the number and sizes of breeding sites in the upper sub-population increased in the course of the study, this phenomenon might be explained by an examination of hydrological data for the area.

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