

# Species and sex ratio differences in mixed populations of hybridogenetic water frogs: The influence of pond features<sup>1</sup>

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**Abstract:** Among closely related species with overlapping fundamental niches, usually one species excludes the other(s) unless they differ in their realized niches. In some instances, however, niche overlap is inevitable. One example is European water frogs of the *Rana lessonae* / *R. ridibunda* / *R. esculenta*-complex, where the hybrid *R. esculenta* is reproductively dependent on one or the other of the two parental species. Hence, it has to live in close sympatry with them, but species and sex ratios in such mixed populations vary widely among ponds. In this paper we investigate the spatial and temporal distribution of the three species by comparing nine ponds with different ecological conditions and frog proportions. All three species occurred in all ponds, but in significantly different absolute and relative numbers. *R. lessonae* proportions are higher in smaller, more structured ponds with rich vegetation under water while *R. ridibunda* dominates in larger, less structured ponds with little vegetation under water. The hybrid *R. esculenta* is intermediate in its distribution; it occurs in larger ponds than *R. lessonae*, but in more vegetated ones than *R. ridibunda*. The sex ratios of the three species differ also within the ponds. In *R. esculenta*, the proportion of females decreased with increasing fluctuation in water temperature. The observed spatial distribution of adults is best explained through species differences in the habitat-related development and survival of their progeny, which is known from several experiments. Temporal changes in species proportions were not related to pond characteristics. This, however, is not surprising, as year-to-year changes in pond features were small, and strong site fidelity, combined with high annual survival, creates substantial temporal autocorrelation between population compositions of successive years.

**Keywords:** species composition, spatial and temporal distribution, ecological conditions, Amphibia, *Rana*.

**Résumé :** Les espèces très proches parentes qui possèdent des niches fondamentales qui se chevauchent s'excluent généralement les unes les autres, à moins que leurs niches réalisées ne soient différentes. Dans certains cas, le chevauchement des niches est toutefois inévitable. On trouve un exemple de ce phénomène chez les grenouilles européennes du complexe *Rana lessonae* / *R. ridibunda* / *R. esculenta*. L'hybride *R. esculenta* dépend pour sa reproduction de l'une ou l'autre des deux espèces parentales. Cet hybride doit d'ailleurs vivre en sympatrie étroite avec ces espèces. Ceci dit, dans les populations où les trois espèces cohabitent, l'importance respective des espèces et des sexes varie grandement d'un étang à l'autre. Dans cette étude, nous examinons la répartition spatiale et temporelle des trois espèces en comparant neuf étangs aux conditions écologiques différentes. Les trois espèces sont présentes dans tous les étangs, mais en nombres absolus et relatifs différents. On trouve davantage de *R. lessonae* dans les petits étangs bien structurés riches en végétation submergée, alors que *R. ridibunda* domine dans les grands étangs moins bien structurés avec peu de végétation. L'hybride *R. esculenta* a une répartition intermédiaire; il habite de plus grands étangs que *R. lessonae*, lesquels sont davantage colonisés par la végétation que ceux fréquentés par *R. ridibunda*. D'autre part, le rapport des sexes chez les trois espèces diffère entre les étangs. Chez *R. esculenta*, on trouve moins de femelles lorsque les fluctuations de température et d'eau augmentent. La répartition spatiale des adultes s'explique par un développement et un taux de survie de la progéniture propres à chaque espèce et qui sont influencés par les caractéristiques de l'habitat. Les changements temporels de l'importance respective de chaque espèce ne sont pas associés aux caractéristiques des étangs. Ce n'est pas surprenant dans la mesure où les caractéristiques des étangs varient peu d'une année à l'autre, où les grenouilles sont très fidèles à leur étang et en raison d'un taux de survie annuel élevé. De telles conditions sont favorables à une autocorrélation temporelle élevée de la composition des populations d'une année à l'autre.

**Mots-clés :** composition des espèces, répartition spatiale et temporelle, conditions écologiques, amphibiens, *Rana*.

## Introduction

The regional distribution and density of organisms is affected by their fitness response to various abiotic and biotic environmental factors, including density of intra- and inter-specific competitors. Due to their descent from a common ancestor, closely related species usually show similar responses to habitat conditions and, hence, overlap of their fundamental niches. The resulting competition leads to dif-

ferentiation of their realized niches or exclusion of one species by the other (Hardin, 1960; Begon, Harper & Townsend, 1996).

In some cases, however, closely related species must coexist because they are strictly dependent on one another. This holds for sperm-dependent parthenogenetic systems (also called gynogenetic systems; Kirkendall & Stenseth, 1990; Lima, Kobak & Vrijenhoek, 1996), cyclic parthenogenetic systems (Bell, 1982), and hybridogenetic systems (Berger, 1977; Graf & Polls-Pelaz, 1989; Tinti, Montovani

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& Scali, 1995). Such systems consist of mixed populations, with one species living as a sexual parasite that requires the gametes of the host for successful reproduction. Since the sexual parasites originally arise from hybridization (Dawley & Bogart, 1989), they show some characteristics of their host species, including habitat requirements. According to the competitive exclusion principle, this should result in instability of mixed populations. In reality, however, gynogenetic and hybridogenetic systems in nature seem to be remarkably stable over time (Moore, 1976; Berger, 1977).

One example of such stability comes from the European water frog complex, consisting of the two genuine species *Rana lessonae* L. (genotype LL) and *R. ridibunda* Pallas (RR) and their associate *R. esculenta* Cam. (LR), originally a hybrid between the two other species, but – as indicated by its scientific name – usually also termed a species. While both parental species can exist in pure populations (see cells 1 and 9 in Table I), hybrids usually cannot (for exceptions, see, Mikulíček & Kotlík, 2001; Rybacki & Berger, 2001). Typically, *R. esculenta* will only persist in mixed populations through repeated mating between the two parental species (*i.e.*, primary hybridisation; cells 3 and 7) and/or through matings between hybrids and parental animals (cells 2 and 4). This latter pathway is due to “hybridogenesis” (Schultz, 1969), a reproductive mode in which the hybrid eliminates one of its parental genomes premeiotically, in north-eastern Europe the R- and in central Europe, including Switzerland, the L-genome. The remaining genome (L in eastern, R in central Europe) is clonally transmitted to eggs and sperm (Berger, 1977, 1983; Graf & Polls-Pelaz, 1989; Günther, 1990). Probably as a result of this clonal transmission, the fully homozygous offspring resulting from LR × LR matings are usually not viable (cell 5; Berger, 1977; Semlitsch & Reyer, 1992a). Only when LR parents belong to different hemiclones, or at least one of them arises from primary hybridization, will the offspring develop normally (Hotz, Beerli & Spolsky, 1992; Vorburger, 2001a,b). In this case, the R genomes are different, either because of independent origin or because deleterious mutations have not yet accumulated. Where these conditions are not fulfilled, *R. esculenta* can only persist when it lives

sympatrically and mates with the respective parental species (in our case *R. lessonae*) to regain the previously eliminated genome.

In such mixed populations of the two species, both interspecific mating combinations (LR × LL and LL × LR; first individual = female, second = male) result in *R. esculenta* offspring (cells 2 and 4), but in different sex ratios. Water frogs have an XX/XY sex determining mechanism, with males being the heterogametic sex. For size-related reasons, primary hybridization is usually between RR-females and LL-males, with the result that exclusion of the L-genome in the hybrid affects the male chromosome set. Therefore, matings between female *R. esculenta* and male *R. lessonae* will lead to a balanced sex ratio among offspring (cell 4), whereas the reverse mating combination produces only daughters (cell 2). For the same reason, matings between *R. esculenta* males and *R. esculenta* or *R. ridibunda* females result in all female offspring (cells 5 and 8; Hotz, Beerli & Spolsky, 1992). These mating outcomes, however, can explain neither why hybrid sex ratios can range from 100% females in some ponds to 100% males in others nor why hybrid proportions in mixed populations can range from 5% to 95% (Berger 1977; Plötner & Grunwald, 1991; Neveu, 1991; Tunner & Heppich, 1992). A number of authors have invoked ecological specialization, but, unfortunately, most of these studies were purely descriptive (Berger, 1977; Blankenhorn, 1977; Heym, 1974; Günther, 1974, 1990), investigated only one species (Sjögren, 1991, 1994), or made no distinction between hybrid and parental animals (Strijbosch, 1979; Blab, 1982; Pavignano, Giacoma & Castellano, 1990; Ildos & Ancona, 1994). Hence, the reasons for the observed differences in population compositions remain unknown.

Previous investigations have revealed:

(i) Typical breeding habitats are small vegetated ponds in the case of *R. lessonae*, larger and less-vegetated water bodies (lakes, gravel pits, and rivers) in the case of *R. ridibunda*, and the whole range of water bodies in the case of *R. esculenta* (Günther, 1990)

(ii) Adults show a high degree of site fidelity (Holenweg Peter, 2001a,b; Holenweg Peter, Reyer & Abt Tietje, 2001).

(iii) In experiments, tadpoles of the three species perform differently under a wide variety of biotic and abiotic factors (Stauffer & Semlitsch, 1993; Horat & Semlitsch, 1994; Plenet *et al.*, 2000; Negovetic *et al.*, 2001).

Based on these findings, we made the following predictions: (1) Species and sex ratios among adult frogs should differ among breeding ponds. (2) Ratios within a pond should be stable over time, both within and between years. (3) Spatial differences and temporal stability are related to ecological parameters. For testing these predictions, we chose a study area where all three species coexist. This was done to eliminate the possibility that differences in species composition merely reflect different geographical distributions of the three species (Günther, 1990; Pagano, Joly & Hotz, 1997). In the discussion, the observed species and sex ratios are compared to previous data on larval performance, mating behavior, and pond-to-pond movement and are interpreted in the light of theoretical models.

TABLE I. Outcome of all possible mating combinations in mixed populations of the two parental species *Rana lessonae* (LL) and *R. ridibunda* (RR) and their hybrid *R. esculenta* (LR and RL). The subscripts *x* and *y* denote the female and male genome, respectively. The bold L in male and female *R. esculenta* indicates that in our study area hybrids exclude the *R. lessonae* genome premeiotically and, hence, produce only eggs and sperm containing the *R. ridibunda* genome. The asterisk in cell 5 illustrates that *R. ridibunda* offspring resulting from hybrid × hybrid matings are usually not viable when the parents belong to the same hemiclone, but develop normally when male and female carry different R genomes and/or those not yet loaded with deleterious mutations. For further explanations, see text.

	MALE	<i>R. lessonae</i>	<i>R. esculenta</i>	<i>R. ridibunda</i>
FEMALE		$L_x L_y$	$R_x L_y$	$R_x R_y$
<i>R. lessonae</i>	$L_x L_x$	$^1 L_x L_x / L_x L_y$	$^2 L_x R_x$	$^3 L_x R_x / L_x R_y$
<i>R. esculenta</i>	$L_x R_x$	$^4 R_x L_x / R_x L_y$	$^5 R_x R_x^*$	$^6 R_x R_x / R_x R_y$
<i>R. ridibunda</i>	$R_x R_x$	$^7 R_x L_x / R_x L_y$	$^8 R_x R_x$	$^9 R_x R_x / R_x R_y$

## Methods

### STUDY AREA

During two years (1995 and 1996), we investigated a total of nine different breeding ponds (1-6, A-C) in a military training field near Zürich Airport, Switzerland. The area was chosen for three reasons. First, it provides the range of different pond types and ecological characteristics that is necessary for testing the three predictions outlined in the introduction (Table II). Second, all ponds are located in an area of 2 km<sup>2</sup> with distances of 100-1,200 m between them. Since water frogs can easily move over such and even larger distances (Tunner, 1992; Holenweg & Reyer, 2000; Holenweg Peter, 2001a), we assume that in our population they can choose a particular pond over others. Third, the area contains not only the typical mixed *R. lessonae*/*R. esculenta* population, but also *R. ridibunda*, which probably was introduced via the nearby airport (Grossenbacher, 1988). This sympatry in a small but ecologically variable area allowed us to monitor the habitat requirements of all three species in the system unconfounded by geographical distribution and to directly compare the breeding habitat of the hybrid with that of its parental species. Further details on the study area are given by Holenweg Peter (2001a,b), Holenweg and Reyer (2000), and Holenweg Peter, Reyer and Abt Tietje (2001).

### COMPOSITION OF MIXED WATER FROG POPULATIONS

To measure species and sex ratios, we collected frogs from all ponds, but in different ways. Pond 1 was surrounded by a drift fence equipped with pitfall traps (Gibbons & Semlitsch, 1982) on both sides. The traps were checked daily between 1 May and 10 August of 1995 and 1996. During the same time periods, the unfenced ponds 2-6 were visited every second week at night to sample as many frogs as possible. For catching we dazzled the frogs with a strong flashlight and then grabbed them by hand. The same technique was used at the unfenced ponds A-C, but at these ponds we collected only once between 12 June and 15 July 1996. All frogs were sexed by the presence or absence of vocal sac openings and determined to species, mostly via albumin electrophoresis (Tunner, 1973) because morphological parameters overlap in these two species (Pagano & Joly, 1999). Animals with a snout-vent-length (SVL) > 40 mm were individually marked with PIT tags (Trovan ID 100, Pameda AG, Switzerland). We did not use this

marking technique for smaller animals because the PIT tag (11 × 2 mm, 0.1 g) would have been too big for their body size, and the thin skin of small frogs might have ruptured when inserting the transponder. After being marked, the animals were released either onto the opposite side of the fence (pond 1) or back into the water (all other ponds).

### HABITAT TYPES

To determine the habitat characteristics for each of the nine ponds where frogs were collected, we examined the following features: surface area, volume, segmentation, vegetation structure, and water temperature.

### SURFACE AREA

Pond surface areas were calculated from those geometrical forms that matched the pond shape best (ellipse, rectangle). For ponds 1-6, the dimensions needed for these calculations were determined twice in 1995 and three times in 1996, in both years once when the water level was high and once when it was low to account for changes in pond size. The water levels of ponds A, B, and C did not change much. Their surface areas were determined just once in July 1996 when the other six ponds had an intermediate size.

### VOLUME

The water volume of each pond was estimated by multiplying the mean surface area by the mean maximum depth. In both years, water depth in ponds 1-6 was recorded every second week between 1 May and 10 August with the help of a surveyor's rod. In 1996, we also recorded depth profiles at a time of the year when all nine ponds were of intermediate size. For this profile, depth was measured every meter along parallel transects that were laid over the pond. The distance between transects was 6 m for the larger ponds (4, 5, 6, A, B, C) and 4, 3, and 2 m, respectively, for the smaller ponds 2, 1, and 3.

### SEGMENTATION

An index of segmentation was obtained by dividing the mean length of the shoreline by the mean pond surface area. The higher this ratio the longer is the shoreline compared to surface area, *i.e.*, the more segmented is the pond. The length of the shoreline was determined with the help of a measuring tape that was laid around each pond. These measurements were only taken in 1996, three times for ponds 1-6 and once for ponds A, B, and C.

TABLE II. Ecological characteristics of the nine ponds (1-6 and A-C) that were investigated in this study. Shown are values for 1996, the year with the most complete data set. For the first six ponds, volume is also given for 1995 to illustrate that conditions were similar within ponds between years (temporal stability), but differed among ponds within years (spatial diversity).

Pond no.	Pond type	Volume (m <sup>3</sup> ) 1995	Volume (m <sup>3</sup> ) 1996	Surface area (m <sup>2</sup> )	Segmentation	Vegetation (%) under water	Vegetation (%) above water	Open water (%)	Temperature fluctuation (°C)
1	Peat bog	80	103	100	0.53	54	50	19	6.1
2	Vehicle ruts	94	245	547	0.37	38	20	54	10.2
3	Shallow pond	6	8	38	1.15	83	83	0	9.8
4	Vehicle ruts	467	360	900	0.40	65	33	27	9.9
5	Flooded gravel pit	3294	2524	982	0.13	7	16	79	6.8
6	Peat bog	591	284	355	0.40	59	88	5	8.8
A	Broad ditch		224	400	0.28	97	63	0	8.9
B	Flooded gravel pit		3000	1128	0.13	4	21	76	8.3
C	Shallow pond		429	572	0.29	71	13	23	11.0

## VEGETATION

Along the parallel transects described under volume, we also recorded every meter the presence or absence of vegetation under water (0 to 20 cm below the water surface), above water (plants that emerge from the water, such as *Phragmites australis* Steudel, *Schoenoplectus lacustris* Palla, *Typha angustifolia* L.), and open surface. Vegetation under water is particularly important for tadpoles while vegetation above water is potentially more important for adults (e.g., shelter, solar radiation). From these measurements the proportions of the three categories were calculated for each pond. Although both the absolute amount of the vegetation and the proportions represented by the three change during the year, differences among ponds are maintained through the season. Therefore, we determined the vegetation composition only once for each pond between mid-June and July 1996.

## WATER TEMPERATURE

For two reasons, thermal differences between ponds were characterized through temperature fluctuations: First, species and sex ratios are not related to absolute water temperature (Holenweg Peter, unpubl. data). Second, fluctuations in water temperature influence the survival and development of larvae more than small differences in the absolute temperatures (Günther, 1990). Temperature fluctuations were recorded between May and August 1996 with mini-max thermometers hanging 10–20 cm below the water surface at a distance of > 2 m from the shore. We checked the thermometers once a week and calculated the pond specific mean fluctuations from the weekly maximum–minimum differences.

## STATISTICAL ANALYSIS

Our catches resulted in data on species and sex proportions from six ponds (1–6) for two years (1995 and 1996) and from three additional ponds (A, B, C) for one year (1996). In addition, they yielded information on the extent to which individuals moved between these ponds. For each pond, species and sex ratios were calculated from the total number of individually marked frogs caught at that pond. Each frog was only counted once, even when it was caught repeatedly during the season. Since the catching probability did not differ between the species and was higher for males than for females at all ponds (Holenweg Peter, 2001a), pond-to-pond differences in the species and sex ratios cannot be attributed to sampling biases.

In a first step, we tested for spatial differences in species and sex ratios among ponds and for temporal differences among catching dates and years by means of multivariate analyses of variance (MANOVA). Species ratios, and then sex ratios, from individual catching events were related to two factors (pond and year), the interaction of these factors, and two covariables (date of catching and number of caught frogs). For this analysis, we used data from both years, but only from those five ponds (2–6) where we caught frogs several times per season in the same fashion.

In a second step, we related frog compositions to ecological factors. For these analyses, the mean frequencies of species or the mean sex ratios within the species per pond and year were used as dependent variables. For pond 1,

these values were calculated from animals caught at the fence between 1 April and 10 September of 1995 and 1996. For ponds 2 to 6, the means were derived from the biweekly catches between 1 May and 10 August, and for ponds A, B and C they were equated with the single measurements. In spite of these differences in the sampling schedule, we can pool the data from all ponds since survival rates are constant from May to August (Holenweg Peter, 2001a), rates of pond-to-pond dispersal are similar within and outside the breeding season (Holenweg Peter, 2001b), and sampling date does not affect the species and sex ratio (Table III).

To approximate normal distribution, all proportions were arcsine square root transformed, and logarithms were used for pond volume and segmentation. Statistics were calculated on Systat 6.0 for Windows (SPSS Inc., Chicago, Illinois). The specific tests are mentioned with the respective results. All residuals were normally distributed.

## Results

## TEMPORAL AND SPATIAL DIFFERENCES IN FROG COMPOSITION

We caught a total of 3,175 frogs, 1,551 of them in 1995 and 1,624 in 1996. Averaged over both years and all ponds, 47.8% ( $\pm 13.3$ ) of the animals were *R. esculenta*, 36.4% ( $\pm 18.9$ ) were *R. lessonae*, and 15.8% ( $\pm 16.3$ ) were *R. ridibunda*. Mean proportions ( $\pm$  SD) of females within the three species were 63.8% ( $\pm 8.0$ ), 53.1% ( $\pm 14.0$ ), and 74.6% ( $\pm 22.3$ ), respectively. These figures reveal a significant excess of females in *R. esculenta* and *R. ridibunda* (both  $P < 0.005$ ; Wilcoxon test), as opposed to the even sex ratio in *R. lessonae* ( $P = 0.594$ ).

Both sexes and all three species occurred at all 9 ponds, but the fairly large standard deviations given above indicate that frog composition differed markedly in time and/or

TABLE III. Results from two multivariate analyses of variance relating relative numbers (i.e., species ratios) and sex ratios, respectively, of *Rana esculenta*, *R. lessonae*, or *R. ridibunda* per catch and pond to the independent categorical variables pond and year, to the interaction of those variables, and to the covariables date of catch and sample size per catch. Multivariate tests were based on Wilks' Lambda statistics; univariate results are only shown when the multivariate test was significant. Significant results are shown in bold.

Effects	Species ratio			Sex ratio		
	F-ratio	df	P	F-ratio	df	P
POND						
<i>R. esculenta</i>	0.647	4, 62	0.631	<b>4.697</b>	4, 44	<b>0.003</b>
<i>R. lessonae</i>	<b>5.915</b>	<b>4, 62</b>	<b>&lt; 0.001</b>	<b>5.490</b>	4, 44	<b>0.001</b>
<i>R. ridibunda</i>	<b>21.668</b>	<b>4, 62</b>	<b>&lt; 0.001</b>	<b>4.802</b>	4, 44	<b>0.003</b>
Multivariate test	<b>7.307</b>	<b>12, 159</b>	<b>&lt; 0.001</b>	<b>4.720</b>	12, 111	<b>&lt; 0.000</b>
YEAR						
Multivariate test	0.171	3, 60	0.916	2.077	3, 42	0.118
POND • YEAR						
<i>R. esculenta</i>	1.111	4, 62	0.359			
<i>R. lessonae</i>	<b>2.606</b>	<b>4, 62</b>	<b>0.044</b>			
<i>R. ridibunda</i>	<b>3.112</b>	<b>4, 62</b>	<b>0.021</b>			
Multivariate test	<b>2.930</b>	<b>12, 159</b>	<b>0.001</b>	1.046	12, 111	0.413
SAMPLE SIZE						
Multivariate test	1.003	3, 60	0.398	1.125	3, 42	0.350
DATE						
Multivariate test	0.284	3, 60	0.837	1.564	3, 42	0.212



space. Two multivariate analyses of variance (Table III) revealed that the most obvious significant parameter explaining these differences was pond, indicating that overall species composition and sex ratios differed among ponds. In terms of species, the effect is mainly due to pond-to-pond differences in the ratios of *R. lessonae* and *R. ridibunda*, but not due to differences in *R. esculenta* proportions, which were around 50% in all five ponds (Figure 1a). In terms of sex ratios, the variable pond had a significant effect on the proportions of females in all three species (Figure 1b). In *R. lessonae*, sex ratios were even in 4 of the 5 ponds; in the other two species, they were skewed towards females. The extent of the skew differed among ponds, especially for *R. ridibunda*: in the three ponds with the lowest proportions of this species (3, 1, and 6), almost all *R. ridibunda* were female (mean  $\pm$  SD,  $93.2\% \pm 4.8$ ) whereas at the three ponds with higher proportions (2, 4, and 5), only two out of three were ( $66.0\% \pm 12.6$ ).

Species composition also differed between years, but the change was not the same in all ponds, as shown by the significant pond $\times$ year interaction rather than a year effect alone (Table III). At some ponds, the relative frequency of a species increased markedly from 1995 to 1996 (e.g., *R. lessonae* from 9% to 29% at pond 5) whereas, at other

ponds, the proportion of the same species decreased or remained the same (e.g., from 33% to 31% at pond 4). Again, the univariate tests yielded significance for the two parental species, but not for the hybrid.

Neither the sample size per catch nor the catching date within a year had any influence on sex ratio or species ratio (Table III). Hence, it seems appropriate to pool data from all catches per year and use mean species composition and mean sex ratio per pond and year for further analyses.

#### ECOLOGICAL REASONS FOR POND-TO-POND DIFFERENCES IN FROG COMPOSITION

In order to identify the habitat characteristics that might be responsible for the above pond-to-pond differences in frog composition, we related the mean pond-specific species and sex ratios to the six pond variables that were measured in both years. The ponds differed markedly in all these variables. Volumes ranged from 8 to 3,000 m<sup>3</sup>, segmentation indices from 0.13 to 1.15, vegetation under water from 4 to 97%, vegetation above water from 16 to 88%, open water area from 0 to 79%, and mean temperature fluctuations from 6.1 to 11.0°C.

#### MULTIPLE POND FEATURES

Since at least some of these variables are potentially correlated, we first performed a principal component analysis (PCA), based on the data from the 9 ponds measured in 1996. The PCA resulted in two independent factors (Table IV). The first factor, explaining 68.4% of the variance, represents pond structure. Increasing values indicate increasing segmentation and vegetation, both under and above water, and decreasing pond volume and open water surface. For the second factor, explaining 18.3% of the variance, increasing values represent increasing temperature fluctuations and decreasing vegetation above water.

We then related the mean relative species proportions, and then sex ratios, of the three species to the scores of the two factors by means of multiple regression. The only significant relationship that emerged in the multivariate analysis was one between factor 1 and species composition (Table Va). The smaller the value of factor 1 was, i.e., the larger, less segmented, and less vegetated a pond was, the more *R. ridibunda* and the fewer *R. lessonae* we found (Figure 2a). In other words, with increasing pond size and

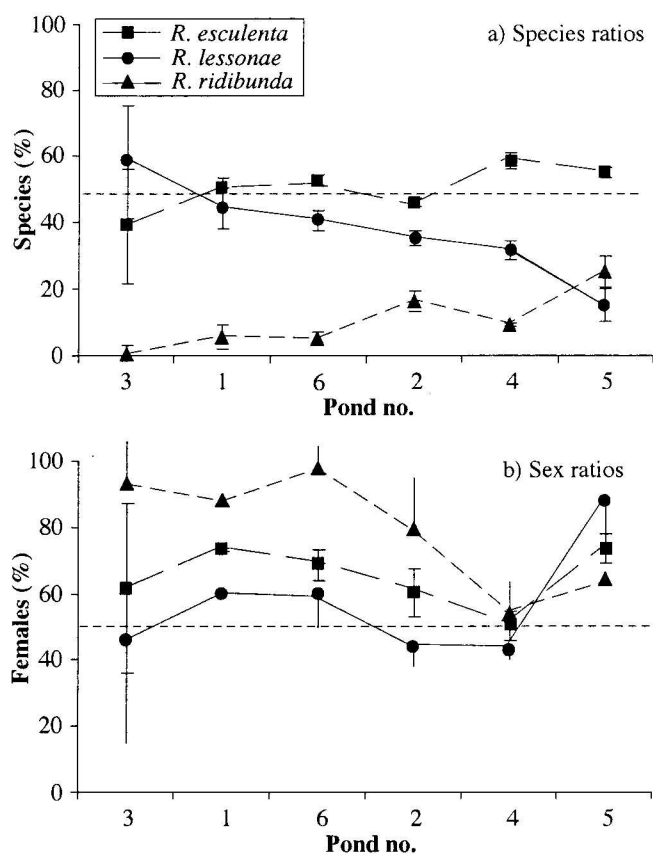


FIGURE 1. Average proportions of (a) species pooled over both sexes and (b) sex ratios within species for the parental species *R. lessonae* and *R. ridibunda* and their hybrid *R. esculenta*. Shown are least square means from the MANOVA in Table II for five ponds (2-6) and ordinary means for one pond (1), all sampled in two years (1995 and 1996). Standard errors are so small that they disappear within the symbols. Ponds are plotted in the order of decreasing *R. lessonae* proportions.

TABLE IV. Results from a principal component analysis (PCA), based on six environmental parameters measured at nine ponds in 1996. For extracting the principal components (factors 1 and 2), we used an eigenvalue of 1 and performed varimax rotation. Numbers represent factor loadings, i.e., correlation coefficients between the original variables in the left column and the extracted factors 1 and 2, respectively. Loadings  $> 0.450$  (bold) are considered to be relevant (Aspey & Blankenship, 1977).

	Factor 1	Factor 2
Volume	<b>-0.925</b>	0.060
Segmentation	<b>0.901</b>	-0.035
Open water area	<b>-0.952</b>	0.044
Vegetation under water	<b>0.886</b>	0.212
Vegetation above water	<b>0.796</b>	<b>-0.455</b>
Temperature fluctuation	0.333	<b>0.914</b>
% of total variance explained	<b>68.373</b>	<b>18.255</b>

TABLE V. Results from a multiple regression relating relative numbers (i.e., species ratios) and sex ratios, respectively, of *Rana esculenta*, *R. lessonae*, or *R. ridibunda* per catch and pond to the two factors extracted by the PCA in Table II. Multivariate tests were based on Wilks' lambda statistics. Significant results are shown in bold.

	Species ratio			Sex ratio		
	F-ratio	df	P	F-ratio	df	P
a) EFFECT OF FACTOR 1						
<i>R. esculenta</i>	1.000	1, 6	0.356	2.725	1, 6	0.150
<i>R. lessonae</i>	<b>13.768</b>	<b>1, 6</b>	<b>0.010</b>	0.164	1, 6	0.700
<i>R. ridibunda</i>	<b>6.280</b>	<b>1, 6</b>	<b>0.046</b>	0.541	1, 6	0.490
Multivariate test	<b>9.102</b>	<b>3, 4</b>	<b>0.029</b>	1.359	3, 4	0.375
b) EFFECT OF FACTOR 2						
<i>R. esculenta</i>	0.106	1, 6	0.756	5.116	1, 6	0.064
<i>R. lessonae</i>	0.331	1, 6	0.586	3.060	1, 6	0.131
<i>R. ridibunda</i>	0.331	1, 6	0.586	0.186	1, 6	0.682
Multivariate test	3.254	3, 4	0.142	3.084	3, 4	0.193

decreasing vegetation, the dominant frog type shifts from one parental species (*R. lessonae*) to the other (*R. ridibunda*). The proportion of the hybrid *R. esculenta* was not affected by factor 1, nor was there an overall effect of factor 1 on sex ratio. Factor 2 (temperature fluctuation) influenced neither species composition nor sex ratio in the multivariate analyses, but univariate analyses revealed that in *R. esculenta* female proportions tended to increase with decreasing temperature fluctuation (Table Vb).

#### SINGLE POND FEATURES

The above analysis enables us to predict (at least to a certain extent) species ratios from the overall habitat characteristics of a pond, but it does not reveal which specific ecological factors are the best predictors. Therefore, we tested, with the same data set as above, whether single pond features provide an equally good or even better explanation for the observed spatial differences in species and sex ratios.

Mean species proportions and sex ratios, respectively, were related to the six pond parameters used in the previous analysis by means of stepwise multiple regression. For all three species, only two variables turned out to be important: pond volume and vegetation under water (Table VI). With increasing pond volume, the proportion of *R. esculenta* increased and that of *R. lessonae* decreased (Figure 2b). With increasing vegetation under water, the proportion of *R. esculenta* increased while that of *R. ridibunda* decreased (Figure 2c). Thus, the hybrid seems to resemble *R. ridibunda* in its response to volume (preference for larger ponds), but *R. lessonae* in its response to vegetation under water (preference for more vegetation). This explains why factor 1, which contains these two negatively correlated environmental variables, was not significantly related to the proportion of *R. esculenta* in the multivariate analysis (Table V). Conversely, the close correlation between various ecological parameters can be considered responsible for the finding that the species distribution is significantly influenced by a composite factor (e.g., factor 1 for *R. lessonae* and *R. ridibunda*; Table V), but not necessarily by each of the variables within that factor (e.g., no effect of pond volume on *R. ridibunda* distribution; Table VI).

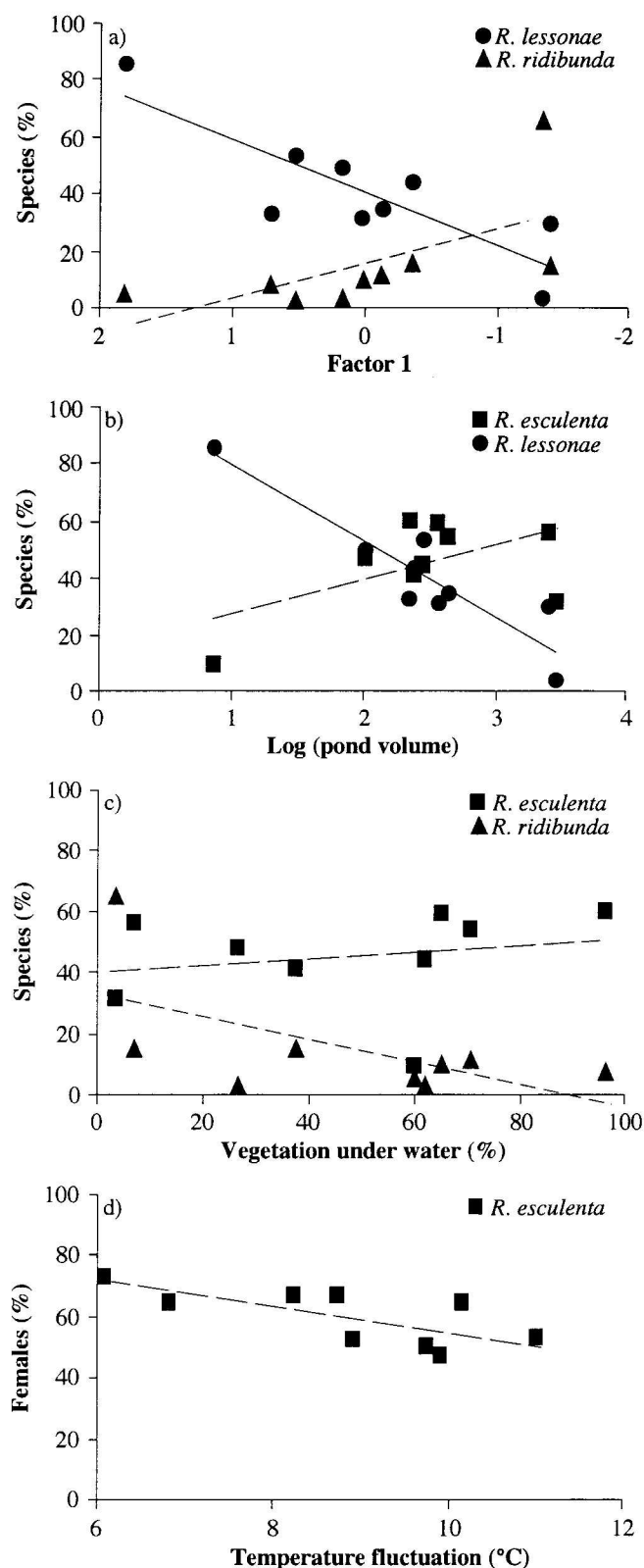


FIGURE 2. Species and sex composition at 9 ponds in 1996 in relation to ecological conditions. Plotted are only those relationships that turned out to be significant in the analyses of Tables IV and V. The first three graphs show relative frequencies of *R. lessonae*, *R. esculenta*, and *R. ridibunda*, pooled over both sexes, relative to (a) factor 1, (b) pond volume (log transformed), and (c) vegetation under water. The last graph (d) shows the proportion of *R. esculenta* females in relation to temperature fluctuation.

TABLE VI. Results from stepwise forward regressions for each of the three water frog species. Dependent variables are average relative numbers (*i.e.*, species ratio) or sex ratios of *Rana esculenta*, *R. lessonae*, or *R. ridibunda* per pond; independent variables were the same as in Table III, namely pond volume, segmentation, open water area, vegetation under water, vegetation above water, and temperature fluctuation. The probabilities for entering and removing variables were 0.05. Shown are only those variables that remained in the final model. SE = standard error, *R* = regression coefficient of the final model.

	Species ratio					Sex ratio				
	Coeff.	SE	F-ratio	P	R	Coeff.	SE	F-ratio	P	R
<i>RANA LESSONAE</i>					0.905					
Volume	-0.309	0.055	31.554	0.001						
<i>RANA ESCULENTA</i>					0.828					0.685
Volume	0.281	0.077	13.12	0.011						
Vegetation under water	0.403	0.153	6.968	0.039						
Temperature fluctuation						-0.042	0.017	6.188	0.042	
<i>RANA RIDIBUNDA</i>					0.691					
Vegetation under water	-0.416	0.164	6.399	0.039						

The corresponding analyses for sex ratios confirmed results from the analysis with multiple pond features (Table VI). Water temperature fluctuation had no effect on sex ratios of the two parental species, but exerted a slight negative influence on the sex ratio of the hybrid *R. esculenta* (Figure 2d).

#### DIFFERENCES BETWEEN YEARS

Both analyses, the one with multiple and the one with single pond features, are based on data from the 9 ponds sampled in 1996 (Tables V and VI). Since species ratios also differed between 1995 and 1996 (Table III), we further tested with the reduced data set from the 6 ponds sampled in both years whether these annual differences might also result from changes in pond size. There was, however, no significant effect in the multivariate analysis of variance (Wilks' Lambda;  $F_{3,2} = 1.222$ ,  $P = 0.480$ ) and only a small trend for *R. ridibunda* proportions to increase with increasing pond volume in the univariate analysis (univariate  $F_{1,4} = 5.933$ ,  $P = 0.072$ ).

### Discussion

We found both sexes of all three species at all nine investigated ponds. Thus, ecological conditions seemed to be within the range tolerated by females and males of the three *Rana* species. Yet, relative numbers differed among ponds, which supports earlier studies (Berger, 1977; Plötner & Grunwald, 1991; Tunner & Heppich, 1992). Theoretically, such pond-to-pond differences in frog composition can arise through stochastic events (Som, Anholt & Reyer, 2000), but then we would not expect the observed correlation between species- and sex- ratios and particular pond parameters. The proportion of *R. lessonae* was higher in smaller ponds with dense vegetation under water while *R. ridibunda* was more abundant in ponds with little vegetation; *R. esculenta* was somewhat intermediate, with about equally large proportions at all ponds (Figures 1 and 2a).

In contrast to these species and sex ratio differences among ponds within years, there were no corresponding differences within ponds between the two years of investigation. This, however, is not surprising. First, delayed maturation and high annual survival create substantial temporal autocorrelation between the population compositions of succes-

sive years. Second, pond conditions did not change markedly from one year to the next (Table II). While average volumes between the smallest and the largest pond differed by a factor of 415, volumes within ponds varied only by a factor of < 2.6 (average 1.6). According to Figure 2b, such small year-to-year changes are unlikely to alter the species ratio markedly.

Our finding that the hybrid occurs over a wider range of ecological conditions than both its parental species is consistent with previous qualitative studies suggesting that, on a larger geographic scale, mixed populations of the hybrid and either one or the other of its parental species are more common than sympatry between the two parental species or among all three species (Blankenhorn, 1973; Heym, 1974; Wijnands, 1979; Tunner, 1980; Günther, 1990). Our result that year-to-year variation is low is also consistent with previous studies, which have shown that in many ponds species ratios remain fairly stable over even longer times than the two years considered here (Blankenhorn, 1973, 1977; Berger, 1977). Below, we discuss two potential reasons for such pond-type-related spatial differences and temporal stability in frog composition: (1) larval performance and (2) terrestrial dispersal.

#### LARVAL PERFORMANCE

Most authors agree that population regulation in pond breeding amphibians mainly occurs during the larval period (Wilbur, 1980; Smith, 1983; Berven, 1990). In fact, with respect to the two ecological parameters that turned out to be most important (pond size and vegetation under water), the pond-to-pond differences in species proportions that we found among adults seem to already exist at the larval stage. Based on his field samples from more than 30 ponds, including the ones monitored in this study, J. van Buskirk (unpubl. data) found that relative numbers of *R. lessonae* tadpoles decreased with increasing surface area in two out of three years. This parallels the decrease in *R. lessonae* adult proportion with increasing pond size (Figure 2a,b). And experiments have shown that *R. lessonae* tadpoles are more pelagic, with a preference for vegetation, whereas *R. ridibunda* tadpoles are more benthic and prefer open water (Degani, 1986; Stauffer & Semlitsch, 1993; Horat & Semlitsch, 1994). This parallels the decrease in proportions of adult *R. ridibunda* with increasing vegetation under water

(Figure 2c). The hybrid *R. esculenta* seems to span the whole range of ecological conditions encountered by its parental species (Figure 1), and in most tadpole studies, *R. esculenta* turns out to be a better competitor than the parental species *R. lessonae* and *R. ridibunda* (Semlitsch & Reyer, 1992a; Semlitsch, 1993). This has been attributed to a broader environmental tolerance of the hybrid larvae and adults which might arise from both a general-purpose species and an array of different (hemi)clones, each of which exploits a narrow frozen niche along an environmental gradient (Lynch, 1984; Vrijenhoek, 1984, 1998; Semlitsch, Hotz & Guex, 1997; Hotz *et al.*, 1999; Plenet *et al.*, 2000).

The good agreement between the effects of pond size and vegetation on tadpole performance and adult composition suggests that, to a large extent, adult ratios are determined by mechanisms operating during the larval stage, but the precise nature of these mechanisms remains unknown for natural ponds. This is because almost all data on larval performance in different environments come from tadpoles raised under controlled conditions and with a limited set of environmental factors. In natural ponds, however, complex networks of ecological parameters, some of them not yet even experimentally tested, can modify or even reverse the competitive outcome between species. An illustrative example comes from the effect of predators. Although in experiments *R. lessonae* and *R. esculenta* tadpoles differ markedly in their responses to a variety of vertebrate and invertebrate predators (Semlitsch & Reyer, 1992b; Stauffer & Semlitsch, 1993; Horat & Semlitsch, 1994), their proportions in natural ponds were totally unrelated to predator numbers in all three years of the investigation (J. Van Buskirk, unpubl. data). Moreover, in natural ponds, tadpoles of the three species need not be exposed to the same conditions. Rather, they may segregate along environmental gradients and choose microhabitats that are optimal for their development. Negovetic *et al.* (2001), for instance, found such an adaptive segregation with respect to temperature.

#### TERRESTRIAL DISPERSAL

In areas like ours, where pond conditions do not change markedly from one year to the next, site fidelity is probably a good rule of thumb for finding a pond suitable for reproduction. In line with this argument, almost 90% of the surviving adult frogs marked in one year returned to the same breeding pond in the next year (Holenweg Peter, 2001b). Among juveniles, at least 15 out of 83 marked individuals (18%) returned to their home pond after the first winter (G. Abt Tietje, unpubl. data). Assuming a juvenile survival rate until next spring of about 33% (Altwegg, 2001), this represents a return rate to the home pond of at least 67%. Although lower than in adults, the figure indicates that site fidelity is high even in juveniles.

Among those adults that did change ponds within and/or between years, Holenweg Peter (2001b) found the following dispersal pattern: Pooled over the two ponds with the highest proportion of *R. lessonae* (ponds 3 and 1; Figure 1), the immigrants-to-emigrants ratio was 40:28 in *R. lessonae*, but 26:37 in *R. esculenta*. Conversely, in the two ponds with the highest proportions of *R. esculenta* and *R. ridibunda* (ponds 4 and 5), immigration exceeded emigration

in *R. esculenta* (42:26), but not in *R. lessonae* (24:25). In *R. ridibunda*, migrant numbers were too small to be included in this comparison. Thus, if adults change ponds, they tend to move to a pond that is characteristic for their species, *i.e.*, with conditions improving performance of their respective larvae (ecological dispersal; Hellriegel & Reyer, 2000). These species-specific dispersal patterns also suggests that movements are unlikely to reflect a response to overall frog density at a pond.

Another criterion for adult selection of breeding ponds could be the search for suitable mating partners, *i.e.*, conspecifics in the case of the two parental species and heterospecifics in the case of the hybrid. Such reproductive dispersal (Hellriegel & Reyer, 2000) would be consistent with results from female choice experiments (Abt & Reyer, 1993; Reyer, Frei & Som, 1999; Roesli & Reyer, 2000; Engeler & Reyer, 2001) and could theoretically explain why the distribution of the sexual parasite *R. esculenta* overlaps with that of both its potential sexual hosts, *R. lessonae* and *R. ridibunda*, whereas the two parental species occupy different habitats (Figures 1 and 2a). This hypothesis, however, would have predicted that hybrid females preferentially move to ponds with large numbers of parental males and hybrid males to ponds with parental females. Moreover, it would have predicted positive correlations between hybrid female and parental male proportions on the one hand and between hybrid male and parental female proportions on the other. In reality, however, none of these correlations did exist (all  $r < 0.506$ , all  $P > 0.326$ ,  $n = 15$  [6 ponds in 1995 plus 9 ponds in 1996]).

The significantly female-biased sex ratio in *R. esculenta* and *R. ridibunda* (Figure 1) should be viewed as a result of, rather than a strategy for, mating. In our study area, primary hybridization between native *R. lessonae* and introduced *R. ridibunda* does occur, and many matings between hybrids produce viable offspring (C. Vorburger, unpubl. data). Thus, 3 out of the 8 mating combinations resulting in *R. esculenta* and *R. ridibunda* offspring, respectively, produce daughters only (Table I). For these two species, the significant pond-to-pond differences in sex ratio (Table III) may be related to differences in the mating pattern: in ponds 3, 1, and 6 the few *R. ridibunda* probably originate from mating combinations involving *R. esculenta* males (cells 5 and 8 in Table I), which produce females only (Figure 1). In contrast, the higher proportions of *R. ridibunda* in ponds 2, 4, and 5 also enable matings between RR-males and RR- or LR-females, which result in offspring of both sexes (cells 6 and 9). The significant pond effect on *R. lessonae* sex ratios results from just a single pond (pond 5) whereas all others show the even sex ratio that is expected for a sexually reproducing species. Since pond 5 is not a typical habitat for this species (Figure 1), it may be that the females found here are those that avoid breeding ponds because they skip a reproductive season, which is not unusual in water frogs (Sjögren, 1988; Bättig, 1998; Wälti, 2001).

Taken together, our results and the arguments presented above strongly suggest that the different proportions of *R. lessonae*, *R. esculenta*, and *R. ridibunda* that we found among adults from different ponds might be causally related to larval performance, directly through habitat-specific dif-



ferences in tadpole development and survival and/or indirectly because adults select ponds with ecological conditions that are favourable for their respective offspring. This is consistent with the usual claim that amphibian populations are mainly regulated through performance during the larval stage (Wilbur, 1980; Smith, 1983; Berven, 1990). However, recent theoretical models have shown that additional regulation during other stages can decisively and unpredictably modify the population structure and dynamics (Hellriegel, 2000). For the water frog complex, type and stability of various hybrid/parental ratios are decisively affected by a complex combination of the mating pattern (Som, Anholt & Reyer, 2000), female fecundity, larval competitive abilities, and the type of dispersal (*i.e.*, ecological or reproductive; Hellriegel & Reyer, 2000). These factors and their interactions have to be further investigated empirically before we can say to which parameters the composition of mixed water frog populations is most sensitive. These studies will also shed light on the dynamics operating in other tightly coupled systems.

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