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Natural density regulation in tadpoles of the moor frog *Rana arvalis* – preliminary report of a field experiment –

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Introduction

An old key question in ecology concerns the importance of intraspecific competition in the regulation of animal numbers (LACK 1954). In a sufficiently stable biotic environment there is evidence that this may be an important factor determining population size. An extra dimension is added to the question when animals if complex life cycles are considered (WILBUR 1980, WERNER 1988). If intraspecific competition is present, at which stage does it operate?

For frogs, a large number of studies of tadpoles under artifical or seminatural conditions have shown the potential of intraspecific competition for regulating frog populations. Almost invariably, a sufficiently large increased density of tadpoles in experimental containers has been found to increase mortality and/or decrease growth rate (WILBUR 1976, MURRAY 1990, GRIFFITHS 1991). The first does of course directly affect population numbers and may, in the absence of compensating factors, affect the breeding population. The second effect may indirectly affect subsequent survival and population number (SMITH 1987, SCOTT 1994).

So far, few studies have studied density effects in natural populations of amphibians (examples include CALEF [1973], BUSKIRK & SMITH [1991], and SCOTT [1994]). The purpose of the experiment reported on here is to determine whether tadpoles of the moor frog *Rana arvalis* which are living at natural densities in their natal pond are affected by changes in population density. "Affected" refers to survival and other aspects that may be important for future survival or reproductive performance. This would imply that elements of intraspecific competition are regulating the poplation through the tadpole stage. From a practical point of view, no effect would mean that if it were possible to increase the breeding population, this would result in a corresponding increase in the recruitment. On the other hand, if effects are detected, it is possible that an increase in the habitat quality (and quantity) for postmetamorphic frogs would not, in the long run, lead to a corresponding increase in the population size.

The following report, first presented as a poster at the SEH meeting 1995, is a preliminary report of findings from an ongoing study. Only data from the period 1990 to 1994 are presented here. Data from 1995 have also been collected and the field work is planned to continue for two more seasons. As will be obvious later, this type of study requires data from several seasons to produce useful data. Also, only results concerning the performance of young and middle aged tadpoles will be presented. The full study is also concerned with the performance up to metamorphosis.

Methods

Study animal and study pond: The study is concerned with the moor frog, which belongs to the group of brown frogs in the genus *Rana*. It breeds explosively in spring. In the study area breeding usually takes place during one of the first two weeks in April. Adult frogs breed for the first time at two or three years of age (LOMAN 1978). Spawn is deposited collectively. Groups of spawn may contain up to 100 clumps, rarely even more. Single spawn clumps are also found. There may be several breeding sites in a single pond. Breeding at one site usually lasts for less than 3 days. Hatching takes place after 10 to 20 days, depending on the temperature.

The study pond is situated in the Revinge area, a military training field in southernmost Sweden (55^0 40' N, 13^0 30' E). The study pond is about 10 x 100 m in area. Part of it is overgrown by *Salix*. The rest of the pond has a luxuriant growth of submerged and emergent plants like marsh cinquefoil (*Comarum palustre*), bullrush (*Typha latifolia*) etc. As will be discussed further below, the pond is temporary and dries up completely in most summers. Following warm and dry springs this will take place before the time for tadpole metamorhposis.

In addition to moor frogs, common frogs *Rana temporaria* were also breeding in small numbers in the pond (tab. 1). There were ample populations of predators in the pond. Among the more common were newts, *Triturus vulgaris* and *Triturus cristatus*, larvae and imagines of Dytiscidae (e.g. *Dytiscus marginalis*), and leeches (*Haemopis sanguisuga*). Quantitative measurements were taken of these. These results will not be presented here.

	1990	1991	1992	1993	1994	1995
Rana arvalis	184	108	48	202	139	69
Rana temporaria	21	0	24	15	14	5

Tab. 1. Number of spawn clumps depositied in the ponds by moorfrogs and common frogs.

The pond is typical for breeding ponds of moor frogs in southern Sweden. Most breed in shallow ponds that more or less frequently dry up in the summer. It is common for them to breed together with common frogs.

Design of the experiment: Before the start of the experiment, the pond was physically subdivided into 4 similar sections, from east to west designated A, B, C, and D. The division was done with a plastic sheet that reached just above the water level in spring. It was not possible for tadpoles to pass through the sheet. Total conformity between the sections could of course not be guaranteed but the procedure and analysis adopted were designed compensate for this. However, in particular A, D and B, C respectively, were pairwise quite similar with respect to size and vegetation. The predatory inventory also gave similar results for all sections.

After breeding, all spawn deposited in the pond was redistributed into four new groups of spawn, one in each section. The relative amount of spawn in the four sections was after this 1 : 4 : 1 : 4. After that, the eggs were left to hatch naturally and the tadpoles grew in their respective sections, at the densities determined by the experimental manipulation and, following that, natural mortality in the sections. The procedure was repeated for 5 years (1990 – 1994). 1990, 1992 and 1994 sections A and C were used as high density sections and B and D low density sections. In the years between, the section categories were reversed.

There were two reasons to perform the experiment for several years, apart from the benefit of increasing the sample size. First, it is possible, not to say likely, that the effect of increasing tadpole density depends on the original density. A study spanning over several years is likely to give examples of natural low and natural high density years. The latter hopefully approaches the limit set by natural regulation ("K" in the conventional models based on the logistic equation) - whichever the stage it operates on. The second reason is the necessity to compensate for the inevitable difference in the properties of the four sections. Without between year variation two years would suffice. However, it might be that in a dry year section A is superior to D while in a wet year the reverse is true. Performing the experiment for several years reduces the lack of precision in the response that is due to such conditions. Complete correction for all factors that might interfere with the planned design of the experiment is not possible but such are the terms of a field experiment.

Spawn of common frog, which amounted to less than 10 % of that of moor frogs, was treated in the same way as that of moor frogs. These species have a similar ecology and probably compete (LARDNER 1995). It was therefore felt that leaving the spawn of common frogs in place would in a variable way (different amount of spawn between years and sections) affect the competitive situation for moor frogs and thus introduce an unwanted noise in the analysis. However, if any competitive effects were found with the design chosen, the main part of it would be intraspecific as moor frog tadpoles always dominated heavily.

When deciding on the proportion of spawn in the sections, the following considerations were taken. Too high increase in density above the natural level would not give evidence of the kind of response I am interested in. Effects on the performance of tadpoles after artificially increasing the density 10 fold would not proof that regulating mechanisms operated at the present natural level. Also, too drastic reduction of density in the low density sections would make it difficult to obtain samples of tadpoles for measurement. On the other hand, too small difference between high and low density sections would make it impossible, with reasonable sample sizes, to detect any real differences between the two types of treatment. The proportion 1 : 4 : 1 : 4 was deemed a suitable compromise. This would change an initial average density of e.g. 25 : 25 : 25 : 25 clumps into 10 : 40 : 10 : 40.

Practical procedure: The pond was regularly checked during spring in order to find all spawn deposited. When a breeding site was detected it was left intact for 2 or 3 days to make sure that all egg laying had finished. After that, all spawn (from both *Rana* species) was collected with a sieve. It was weighed and put back in the pond in the determined proportions at four new sites, one in each section. As new breeding sites were found, spawn was likewise collected and redistributed at the same four new sites. These were similar to those where the spawn was originally found.

In the middle of May (interyear variation: 12th to 24th May) and in the beginning of June (3rd to 5th June) samples of tadpoles were taken. In each section 5 to 7 samples, in each period, were taken with a scraper net. The total bottom area covered with each sample was 1 m^2. If very few tadpoles were found, additional non systematic attempts were made to increase the sample for size measurements. Because of the thick bottom vegetation these samples cannot be considered total and do not give evidence of the absolute density. They should be considered an index.

The tadpoles were preserved in 70 % alcohol and later measured and weighed (dry weight). Measures included body length, total length, and hind leg length. In this report only body length will be considered.

At the time of metamorphosis, samples of metamorphs were caught on the shore by hand, measured and released. Apart from the size measurement this gave information for time for metamorphosis. This was considered to have taken place on the day the tail was between 2 and 5 % of body length. However, data on metamorphs is not considered further in this report.

Deposited spawn: The natural density variation is the background against which the following results should be judged. Total amount of spawn varied between 72 and 217 clumps per year, most of them from moorfrogs (tab. 1).

Spring weather and pond area: Following dry and warm springs, the pond dried up before time of metamorphosis, or even before the time for the planned second tadpole sample (fig. 1). Between year differences in drying up rate affected, during the latter part of tadpole development, the actual density as much as did variations in spawn deposited. Because bottom topgraphy was not identical between the four sections, this also in some years resulted in the perturberation of the planned relative densitites.

However, these variations usually had little effect on the May densities. Variation was higher in June but note that tadpoles captured in June had spent most of their growth at densities determined by the experimental design and non weather dependent factors. Only during the last one or two weeks (between the May and June sample times) in some years had drought had an differential influence on density and survival. On the other hand, growth during this period was quick. The average body length of moor frog tadpoles in the May samples was 8.4 mm and in the June samples 10.6 mm. This corresponds to a 2-fold increase in weight. The relevance of the June measurements therefore remains under question in the dry years.

Results

Density: In May, density differences between the high and low density sections were, not surprisingly, significant (2-way ANOVA with factors year and section type (high/low) $P = 0.027$) (fig. 2). Also the effect of year was significant ($P = 0.003$) but not so the interaction between year and section type ($P = 0.077$). On average the density in the high density sections were in May 4.8 times as high as those in the low density sections. This was computed in each year by taking the average of the two high density sections and dividing this by the average of the two low density sections. These quotients were then averaged.

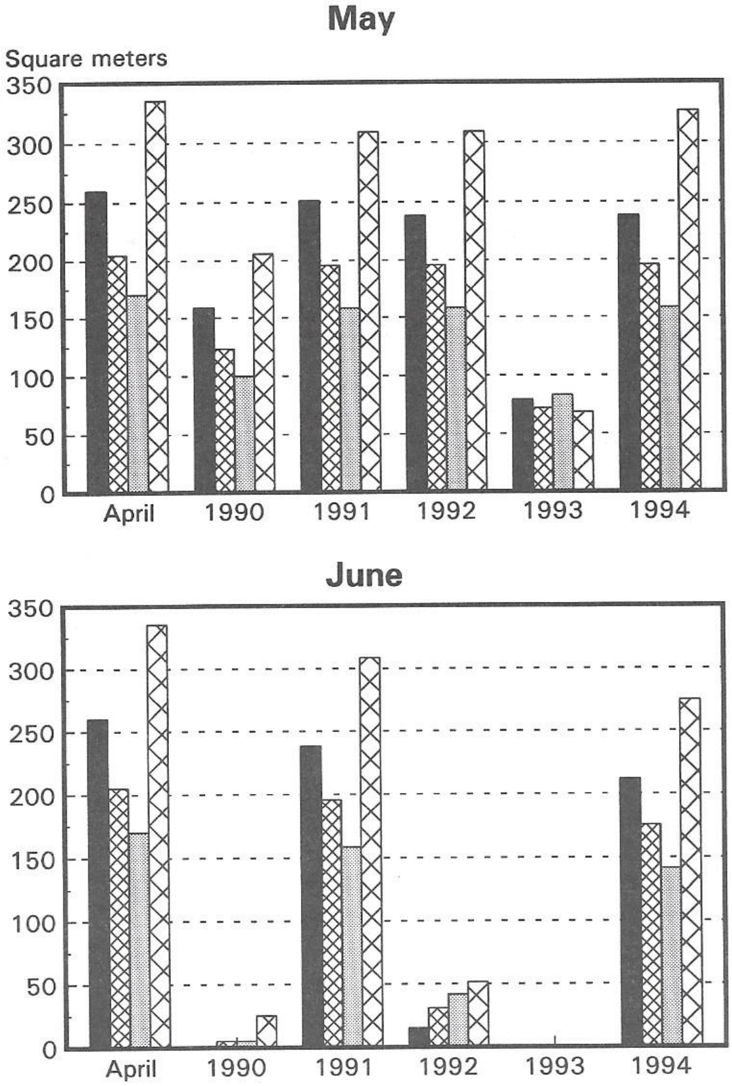

Fig. 1. Remaining pond area at the time of tadpole sampling. The four bars in each cluster represent the sections, A, B, C, and D. At the time of spawning in April, the pond area was similar in all years. This area is indicated in the leftmost cluster. In 1993, the pond was completely dry before the second sample was sceduled.

In June, the average density difference was even larger, being 6.5 times higher in the high density sections. However, possibly due to the perturbations caused by drying up in some years, this was irregular and not significant (2-way ANOVA based on log transformed tadpole density indices, $P = 0.25$). Also, neither year nor the interaction between year and density treatment had a significant effect on tadpole density.

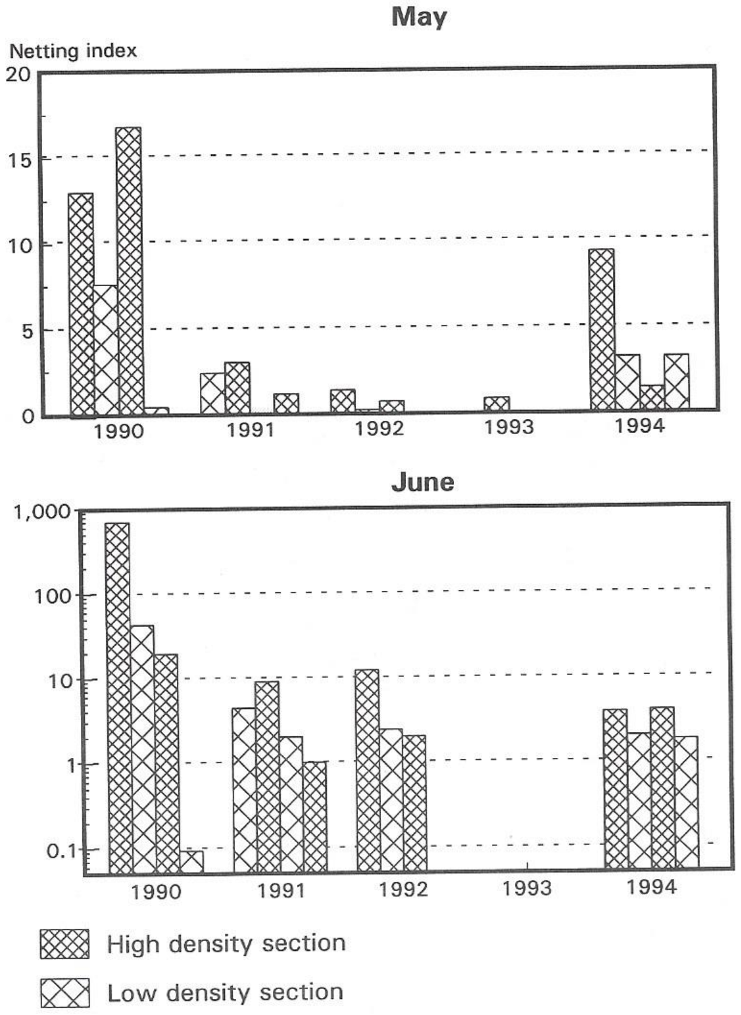

Fig. 2. Tadpole density in May and early June. The index represent the number of tadpoles captured in each netting sample, a fraction of all tadpoles present over 1 m^2 of pond bottom. The four bars in each cluster represent the sections, A, B, C, and D.

The extremely high densities in some sections in June 1990 were due to the drought that concentrated tadpoles in a few remaining puddles.

Summarizing, the relocation of tadpoles had a lasting effect on the relative density in the respective sections. Although the density in some years seemed to converge, this was not significant and not more likely in years with a high average density. This had been indicative of a regulating mechanims operating directly on tadpole survival.

Growth: In May 1990 and 1994 tadpoles in low density sections were larger than those in high density sections (fig. 3). In 1991 and 1992 this was not so. This may be due to the small sample size in these years or that the low density in these years was not sufficient to cause competition resulting in different growth rate between sections. In May 1993 there were no tadpoles at all found in the low density sections. As a consequence, there was

Effect	d.f.	F	P
May			
Year	3	35.8	< 0.001
Density	1	0.8	0.38
Year x Dens.	3	10.0	< 0.001
June			
Year	3	38.1	< 0.001
Density	1	44.6	< 0.001
Year x Dens.	3	7.8	< 0.001

Tab. 2. ANOVA test of factors affecting tadpole body size.

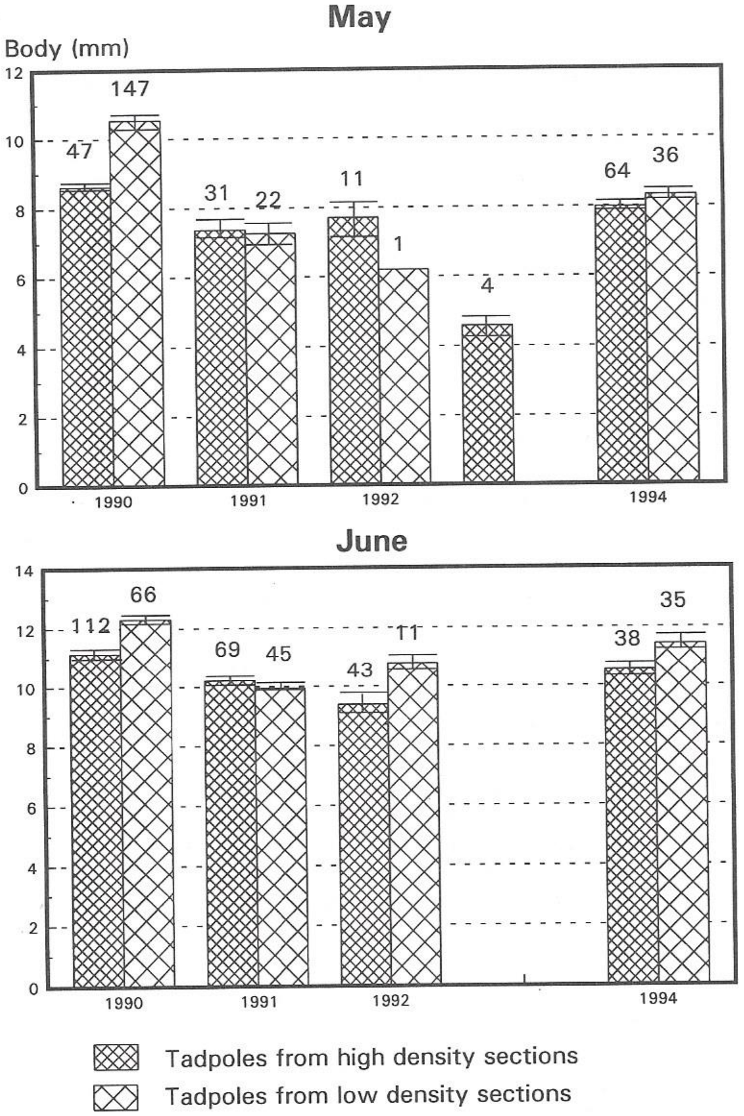

Fig. 3. Average body size of tadpoles captured in low and high density sections. The figures above the bars give the number of tadpoles used. The error bars indicate one mean error above and below mean.

no over all effect of treatment on body size (tab. 2). However, the interaction between year and treatment was significant. This makes it permissable to state that there was in some years an effect of the density treatment on tadpole size in May. In particular, the years with a size difference were those with the highest May density, 1990 and 1994 (fig. 2).

In June, tadpoles in low density sections were always of similar size or larger than those in the high density sections (fig. 2). At this time the over all treatment effect was significant (tab. 2).

In both periods, the year effects were significant.

Discussion

The results suggest that at least in years with high initial density of spawn, there is a density effect on the growth of tadpoles. I have no data that indicate what effects this size difference may have on the survival and growth of the metamorphosed frogs. Thus I am not able to definitely state whether this population is close to its carrying capacity. However, the study demonstrates that regulating effects do start to operate on tadpoles within the normal density range in this population. The results cannot directly be extrapolated to all other populations but must be regarded as a case study. In other populations with more beneficial conditions for adult frogs, tadpole densities may be much higher which is likely to cause more drastic regulating effects. Oppositely, other populations may have mechanisms that limit the adult populations to much lower numbers, in these populations no competitive effects on tadpoles are likely.

As stated, few other studies have used the approach similar to the one taken here. However, based on field experiments in natural ponds with densities in the range of those occurring naturally, VAN BUSKIRK & SMITH (1991) (*Ambystoma laterale*) and SCOTT (1994) (*Ambystoma opacum*) did find density effects on growth and condition (lipid stores) respectively. On the other hand, CALEF (1973) (*Rana aurora*) did not find any definite effect. This is consistent with my suggestion that there will be much variation between species, populations and years in this respect.

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