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Does pond quality limit frogs *Rana arvalis* and *Rana temporaria* in agricultural landscapes? A field experiment

JON LOMAN and BJÖRN LARDNER
Department Animal Ecology, Lund University, SE-223 62 Lund, Sweden

Summary

1. When assessing the cause of population absence or decline, it is important to understand the relative effect of local, regional and global factors. In this study we evaluated the first of these factors for two frog populations.
2. Amphibians are often absent from intensively farmed areas. There could be several reasons for this, one of them being the quality of the aquatic habitat available for reproduction.
3. In order to test why common frogs *Rana temporaria* and moor frogs *Rana arvalis* are absent from most ponds in the intensively agricultural areas of southern Sweden, we performed a field experiment. Spawn of both species were introduced into 18 ponds surrounded by intensively cropped fields.
4. Tadpole performance generally did not differ from that in a set of reference ponds in various other habitat types where one or both of these frog species occurs naturally.
5. In the same experimental ponds and in a number of reference ponds, we also introduced tadpoles of the two species into enclosures that protected them from predation and thus increased recapture rate. This experiment revealed that the water quality of farmland ponds is rarely unsuitable for successful frog reproduction.
6. Having measured abiotic and biotic variables in the experimental and reference ponds, we assessed the importance of different parameters to tadpole performance. While farmland ponds generally had higher pH, higher conductivity and higher nitrate and nitrite concentrations than our reference ponds, these factors had no discernible effects on tadpole performance under the ranges found across all pond types. None of the other parameters differed between the two groups of ponds, nor did they have any strong or obvious effects on tadpole performance or survival.
7. *Synthesis and applications*. The results indicate that water quality alone is not responsible for the scarcity of amphibians in farmland areas of southern Sweden. To understand better the cause of their rarity, future studies should also focus on the quality of the terrestrial habitat surrounding the ponds and the metapopulation structure.

Key-words: anura, eutrophication, farm land, growth, survival, tadpole, water quality

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Introduction

Anthropogenic habitat alterations often have profound effects on the local distribution of animals. For many habitat specialists, these effects are invariably negative and, although some species can benefit from human disturbance (Banks 2004), an optimum disturbance level usually exists. Above this, the human landscape becomes increasingly sterile and less diverse.

Agricultural landscapes involving arable land are the dominant habitat in many regions. Most frogs depend on both aquatic and terrestrial habitats for their survival, and frog diversity is typically much lower in agricultural areas than in surrounding habitats (Bonin *et al*. 1997; Lehtinen, Galatowitsch & Tester 1999).

Although areas with intensive agriculture are rarely optimal for amphibians, the common frog *Rana temporaria* L. is occasionally found in the agricultural landscape of Scania, the southernmost province of Sweden (Loman 2005). While the moor frog *Rana arvalis* Nilsson has an ecology very similar to that of the *R. temporaria* (Loman 1979) and often breeds in the same ponds (Loman 2004), it is very rarely found in the intensively

Correspondence: Jon Loman, Department Animal Ecology, Lund University, SE-223 62 Lund, Sweden
(e-mail jon.loman@zooekol.lu.se).

Table 1. Overview of ponds in the study. All ponds in study year refers to the year(s) when measurements were taken and, for experimental areas only, when spawn and tadpoles were introduced. All ponds were monitored for frog breeding in both 2003 and 2004

Habitat	Species breeding	Area name	Study year	Number of study ponds (of these used for spawn introduction)	Additional breeding survey ponds
Reference ponds					
Mixed	*R.a.* and *R.t.*	Revinge	03, 04	4	
Forest	*R.t.*	Harphult	03, 04	2	
Bog	*R.a.* and *R.t.*	Gillastig	03, 04	1	
		Arup	03, 04	1	
Agricultural	*R.t.*	Harlösa	03, 04	2	
		Skarhult	03, 04	2	
Experimental areas					
Agricultural	*R.t.**	Härslöv	03	2 (1)	5
Agricultural	None	Remmarlöv	03	9 (4)	12
Agricultural	None	Rosenhäll	03	6 (3)	7
Agricultural	None	Borgeby	04	5 (3)	3
Agricultural	None	Igellösa	04	6 (3)	6
Agricultural	None	Svenstorp	04	7 (4)	10

*In the Härslöv introduction pond, four naturally laid spawn clumps of *R. temporaria* were found after the spawn introduction.

farmed areas of this province (Berglund 1976; Loman 2005). It is not known which factors are responsible for the rarity of these and many other frog species in extreme agricultural landscapes. Many ponds have been destroyed as part of the agricultural intensification (Agger & Brandt 1988), however, in many agricultural areas of Scania, ponds are present in good numbers, yet few or no frogs breed there. Many studies have linked negative effects to the condition of ponds in agricultural landscapes (reviewed by Bugg & Trenham 2003a,b) and it is possible that those ponds that are present in Scania are unsuitable for frog egg and tadpole development.

Populations may also be regulated by processes affecting the adult or metamorphosed stage (Pope, Fahrig & Merriam 2000; Porej, Micacchion & Hetherington 2004). The extent to which a suitable habitat (such as moist pastures and forests) can support a population may depend both on its area and the distance from suitable breeding ponds (Loman 1990).

The present study focused on testing the first possible explanation: that frogs are rare or lacking in extreme agricultural landscapes in Scania because the ponds are unsuitable for their eggs and tadpoles. First, we investigated whether tadpoles of *R. arvalis* and *R. temporaria* performed worse in ponds in agricultural areas without resident frog populations than in reference ponds with existing frog populations. Secondly, we compared all study ponds with respect to a number of biotic and abiotic variables. Lastly, we investigated relationships between the variance in tadpole performance and biotic and abiotic variables.

Method

STUDY AREAS AND PONDS

Experimental areas

We investigated ponds in six experimental areas in the agricultural landscape of western Scania (see Fig. S1 in the supplementary material) in south Sweden. These areas were chosen to represent a landscape dominated by arable land, with a small fraction of forest, meadows, gardens and farm buildings. The vast majority of ponds in these areas are old marl pits.

In each experimental area data on biotic and abiotic variables were collected in two to nine study ponds (Table 1). Spawn and tadpoles were introduced into one to four ponds in each area. In addition, all ponds within 750 m of these ponds were surveyed for breeding frogs. Only in one case (Hars1) was spawn (*R. temporaria*) found before the start of the experiment.

While not formally a random sample, the selection process was carried out so that the ponds in the experimental areas could be considered a representative sample of ponds in extreme agricultural areas in western Scania.

Reference ponds

A total of 12 reference ponds was included in the study (Table 1). These ponds were known to have supported breeding frogs of either or both study species for at least 10 years (Loman 2004). Two of these ponds represented shallow forest ponds that in many years dried up before the onset of frog metamorphosis. Only *R. temporaria* breeds in these ponds. Two ponds were in peat bog sites with plenty of *Sphagnum* moss and comparatively acid conditions. Both species breed here but in one of them *R. arvalis* dominates heavily. We did not expect to find any agricultural pesticides in these four reference ponds as they were all in forested areas. Four reference ponds were situated in a landscape with a mixture of pasture, moist meadow and moist woodland where both species were known to breed. These four ponds were in a conservation area and no pesticides were used locally. There were also four reference ponds in agricultural areas similar to the experimental areas. These were among the few farmland ponds in Scania where *R. temporaria* is actually breeding although no *R. arvalis* was found in these ponds.

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Table 2. Overview of procedures in and types of measurements taken for different ponds

Category	Caged tadpoles	Introduced spawn	Tadpole, competitor and predator sampling	Metamorph sampling	Algal growth	Water chemistry
Reference ponds						
Mixed	X		X	X	X	X
Forest			X	X*	X	X
Bog			X	X†	X	X
Agricultural			X	X		X
Experimental areas						
Introduction ponds	X	X	X	X	X	X
Other study ponds			X	‡		X

*Only 2004.
†In bogs 2004, sampled metamorphs had sufficiently long tails to estimate size at but not day for metamorphosis.
‡There was no breeding and thus no metamorphs to sample in these ponds.

The reference ponds were not a random sample of ponds with breeding *R. arvalis* and *R. temporaria* but were chosen to represent the range of pond types used by these species in the region.

SPAWN INTRODUCTION EXPERIMENT, FREE-LIVING TADPOLES AND METAMORPHS

We introduced 20 spawn clumps from each of the two species into each of 18 ponds (introduction ponds) in the experimental areas. This was considered sufficient to mimic viable frog populations (see Appendix S1 in the supplementary material for a comparison with spawn density in natural populations). The spawn was collected from two (year 2003) or three (year 2004) source ponds. *Rana arvalis* spawn came from the Revinge area and *R. temporaria* spawn from the Harphult area (see Fig. S1 in the supplementary material). Performance of the introduced animals was monitored by sampling the tadpoles and metamorphs.

CAGED TADPOLE INTRODUCTION EXPERIMENT

The survival of tadpoles hatched from introduced spawn included any direct effects from predation. If predation was heavy, there might be no tadpoles left to catch in some ponds at the time of sampling. To distinguish between effects of predation and poor water quality, we introduced caged tadpoles into the introduction ponds and, as a control, also into four of the reference ponds (Table 2). Eight 10-L buckets were placed in each of these ponds. The buckets were prepared by replacing most of the side with a plastic net that permitted free circulation of water but kept the tadpoles in and predators out. Small stones were placed in the buckets to provide some structure, hiding places and to act as a ballast. A standard amount of dry *Phragmites* stalks was also provided as an additional substrate for epiphytic algae. Floats were attached so the buckets could be left floating in the water, giving some buffer to water level variations. The buckets were covered to prevent avian predation, and a 2-cm free air space was left under the cover.

Eight spawn clumps of each species were hatched and the tadpoles raised to the age of 4 days at Gosner (1960) stage 26 or 27. For each pond, two sibling tadpoles of each species were introduced into each of the eight buckets. Thus the same eight sibships (for each species) were used for all caged introductions in 1 year.

The tadpoles were collected from the containers at the same time as predators and free-living tadpoles were netted (3 June–8 June 2003, 31 May–3 June 2004). Survival (tadpoles per container) was scored, and their size and hindleg development (length) was measured.

VARIABLES MEASURED

Abiotic effect variables

Abiotic pond characteristics were determined for all study ponds in the experimental areas and for the reference ponds (Table 2). Pond area was estimated at the time of spawning and drying rate was measured as the percentage decrease in area until early June. Water temperature and water chemistry variables (O_2, conductivity, pH and ammonium, nitrate and nitrite ion concentrations) were measured in early May and again in late May, and the percentage of the pond perimeter that was shaded by trees and bushes was estimated (details on the estimation of abiotic variables are given in Appendix S1 in the supplementary material).

All physical and chemical measures were subject to a principal components analysis. This produced four principal components (PC) (see Appendix S2 in the supplementary material for details). A high value for PC1 indicates a small pond that is not subject to drying and has a high pH and high conductivity. High values for PC2 indicate cool ponds with much of the shore in shadow. PC3 is correlated with nitrogen loads (nitrate and nitrite). A high value for PC4 indicates low O_2 and high ammonium concentrations in the water.

Biotic effect variables

Growth of periphytic algae was monitored in introduction ponds in the experimental areas and in most

Table 3. Ponds with poor tadpole performance. This table lists all ponds with no survival of free-living tadpoles, i.e. no tadpoles were captured when netting and no metamorphs were captured at searches, equalling a survival score of −1·30 (Fig. 1). In addition, ponds with less than 20% survival in the cages, ponds where tadpoles or metamorphs were very small and ponds with very late metamorphosis are listed. The actual values and limits to those selected can be assessed from outliers in Figs 1–3. Years for introduction ponds are listed in Table 1. All extreme values (high, low) are not listed, only outliers. For listed ponds, extreme environmental values are reported as possible causes for the poor tadpole performance. The limit to qualify for reporting can be assessed from outliers in Figs 4 and 5. Values in parentheses are listed for reason of being outliers only, even if it is not obvious why these values should be detrimental to the tadpoles. Other relevant observations are also listed. Note that lack of listing of a tadpole variable does not imply good performance but may because of lack of data. For example, *R. arvalis* was not breeding in many ponds

	Poor performance for	Abiotic and biotic outliers	Other observations
Introduction ponds			
Borg3	*R.a.* free-living survival	PC3 2·44	Duck feeder*
Borg4	*R.a.* and *R.t.* free-living survival	Algae weight 0.00	
Borg5	*R.a.* and *R.t.* free-living survival	PC4 −2·15	Duck feeder*, O_2 level in late May extremely low, 9·9%†. High number of *B. bufo* larvae‡
Igel1	*R.a.* and *R.t.* free-living survival	Algae weight 0·00	
Sven8	*R.a.* free-living survival and *R.t.* free-living tadpoles small and *R.a.* caged tadpoles small		Rotten sugarbeet in pond§
Sven11	*R.a.* and *R.t.* free-living survival and *R.a*. caged survival		Rotten sugarbeet in pond §, O_2 levels extremely low, 2·7% and 16·5%†
Sven15	*R.t.* metamorphosis late		
Reference ponds			
Agriculture			
Harl8/04	*R.t.* metamorphosis late		High number of *B. bufo* larvae‡
Skar4/04	*R.t.* metamorphosis late	PC2 −1·88	
Mixed			
Rev2/03	*R.a.* caged survival	Algae weight 0·00	Heavily disturbed by cattle tramping¶
Rev8/03	*R.t.* free-living survival	(PC2 1·94), Algae weight 0·00	
Forest			
Harp25/04	*R.t.* metamorphosis late	Algae weight 0·00	
Bog			
Arup3/03	*R.t.* metamorphosis small	Algae weight 0·00	
Gill1/04	*R.sp*. free-living tadpoles small	PC1 −2·56, (PC4 −2·37), Algae weight 0·00	

*Duck feeders were only present in these two ponds.
†These were the only O_2 measurements below 20%.
‡Apart from these two ponds, corresponding numbers of *B. bufo* tadpoles were only found in pond Harl7.
§Rotting sugarbeet was also found in Sven10, however, to a lesser extent than in Sven8 and Sven11.
¶Cattle trampling only occurred in this pond.

reference ponds (Table 2). This was done by introducing small Perspex chambers with two openings covered by a mesh that permitted water circulation but prevented tadpoles and snails from entering and grazing. Four weeks later, standing crop (dry weight) of periphyton was measured. For details on the method see Loman (2001).

Predators were monitored in the study ponds and reference ponds in late May and early June (3 June–8 June 2003, 31 May–3 June 2004). The samples were taken by raking the bottom with a sturdy, triangular dip net. Each sample covered 1 m^2 of the bottom surface. Depending on pond size, three to 10 samples were taken. All invertebrate predators and newts were counted and classified, at least to major groups. Smaller nymphs of Odonata, Notonecta and damselflies were not scored. For the score, these predators were also weighed according to the respective group's or species' estimated predatory impact on tadpoles (see Appendix S1 in the supplementary material for details).

The number of snails (*Lymnea* and *Planorbis*) at least 10 mm in size were counted and used as a measure of competition. The only other species considered to be a possible competitor was *Bufo bufo* (L.) larvae. Presence of *B. bufo* is indicated in Table 3, although it was difficult to quantify because of the very aggregated distribution of these tadpoles. It was also difficult to assess them on a common scale with the snails.

Initial tadpole density, which may be a cause for intrageneric competition, was measured as number of spawn clumps per m^2 water surface at the time of breeding. Both species (*R. arvalis* and *R. temporaria*) were included as their tadpole ecology is similar and they have been shown to compete (Lardner 1995). The number of spawn clumps included those introduced into the ponds in the experimental area.

FREE-LIVING TADPOLE AND METAMORPH PERFORMANCE

Tadpoles were caught by netting them together with predators and competitors. The mean number captured per sample (each covering 1 m^2) provided an index of density. It cannot be considered a true measure of density as the benthic structure (stones, tussocks, etc.) differed among ponds. Each tadpole's body and hind leg were measured. Body length was used as a measure of size. Hind leg length divided by body length was used as a measure of development rate, where a tadpole with relatively long legs was considered closer to metamorphosis than one with short legs.

A tadpole survival index was computed by multiplying the tadpole density index by pond area and dividing this estimated total number of tadpoles by number of spawn clumps laid in or introduced into the pond.

At the time of metamorphosis all study ponds with introduced spawn and most reference ponds (Table 2) were visited regularly. At each visit, we searched the pond shore for metamorphs for 3–10 min, depending on pond size. All metamorphs seen were captured, if possible, and their tail and body length measured. Only frogs with some trace of tail were considered representative of size at metamorphosis. Time for metamorphosis was scored for each individual by correcting the date of capture for size of the tail (Loman 2002a). The average number of metamorphs captured per minute at the three most successful visits to a pond was used to estimate metamorph density. Three visits were used because the number of unsuccessful visits differed greatly among ponds, mainly because it was not possible to foresee when metamorphosis started in each pond. An index of survival to metamorphosis was formed by multiplying the metamorph density by pond perimeter and dividing this index of total number of metamorphs by the number of spawn clumps laid in the pond.

The indices of tadpole survival and survival to metamorphosis were both affected by various errors. For tadpoles, the most obvious was variation as a result of benthic quality and thus catchability of tadpoles. For metamorphs, the most obvious was differences in shore vegetation, affecting detectability and catchability of metamorphs. Fortunately, there was no obvious correlation between these two types of biases expected for a pond. A generalized survival index was therefore formed by computing the first principal component of the two primary indices.

TADPOLE SPECIES IDENTIFICATION

In some of the reference ponds only *R. temporaria* spawn was found so all tadpoles were assumed to be of this species. For the rest of the ponds and for the caged animals, the species of tadpoles had to be determined by examination of dentition and body shape (see Appendix S1 in the supplementary material for details).

DATA TRANSFORMATIONS

The fact that data was taken from most reference ponds in both years, while in others, including all ponds in the experimental areas, data were only taken in 1 year, required special consideration to avoid uncontrolled replication in statistical tests. Where this was a concern, the variables were standardized within year (to mean 0 and SD 1). For those ponds where data for both years were available, the mean of these values was used; for other ponds, the one available value was used. The fact that this removed between-year variation was of no concern because such variation was not part of the scope of the study.

Survival of free-living tadpoles was strongly bimodal, with a group of ponds having no survivors. These data were ranked and the ranks used for the tests. Ammonium, nitrate and nitrite titres were highly skewed and log-transformed before being used in tests.

Results

PERFORMANCE OF TADPOLES IN INTRODUCTION PONDS AND IN REFERENCE PONDS

Survival, growth and development were similar for both species in the introduction and reference ponds (Figs 1–3; see Table S1 in the supplementary material). The only significant effects were for caged *R. temporaria*, where tadpoles in introduction ponds survived better (t-test, d.f. = 20, $P = 0{\cdot}021$; Fig. 1), and for *R. temporaria*, which metamorphosed earlier in introduction than in reference ponds (almost significant, t-test, d.f. = 20, $P = 0{\cdot}051$, Fig. 3) (see Table S1 in the supplementary material). However, it should also be noted that there were a number of outliers with poor performance (Table 3). Poor survival mainly affected introduction ponds, seven out of 18 such ponds were affected but only two out of 12 reference ponds, although the difference was not significant (Fisher exact test, $P = 0{\cdot}25$). All forms of poor performance combined (poor survival plus small or late developed tadpoles and metamorphs) actually affected more reference ponds (58%, seven out of 12) than introduction ponds (50%, nine out of 18).

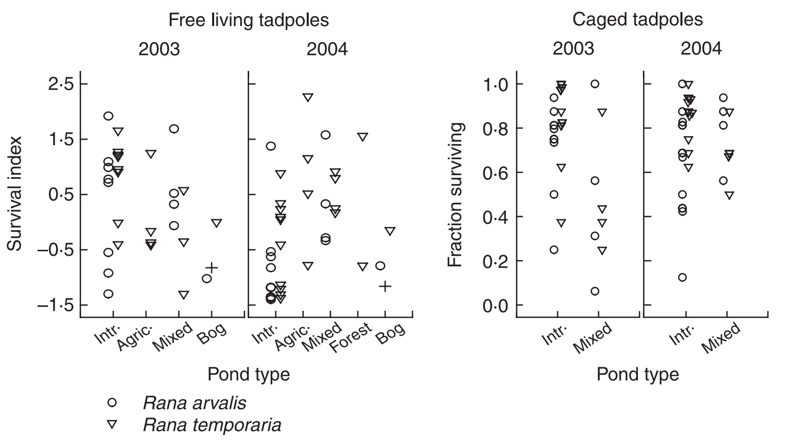

Fig. 1. Tadpole survival. Each symbol represents one species in one pond. Survival for caged tadpoles is shown as the fraction of the eight original tadpoles that survived until the end of the cage experiment. Free-living survival is a generalized index that combines survival of tadpoles until the time for tadpole sampling and the survival until capture of metamorphs. The cluster for free-living survival in 2004 introduction ponds is composed of six *R. arvalis* and five *R. temporaria* symbols. Intr., Introduction; Agric., Agricultural.

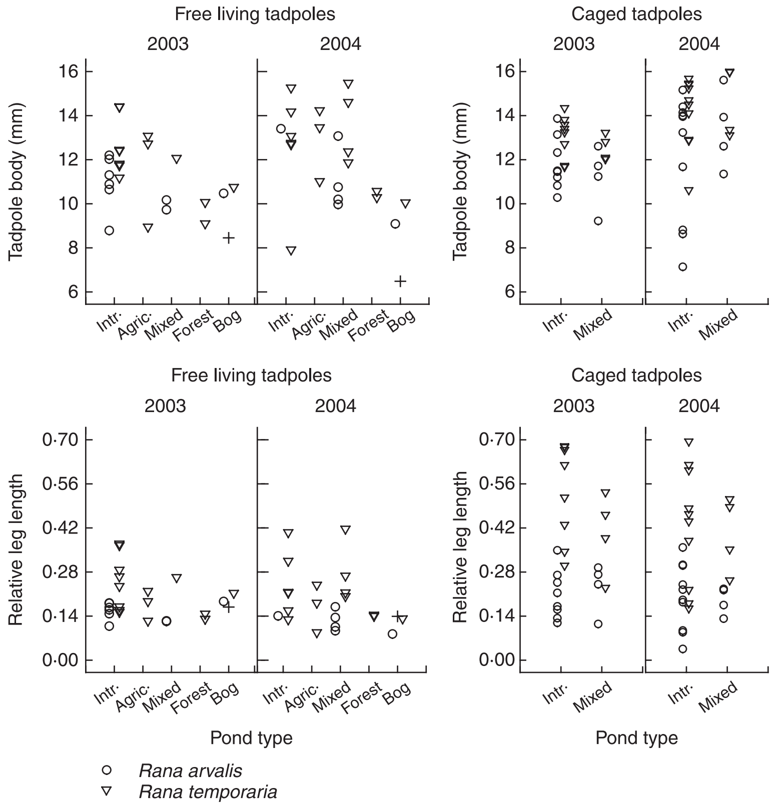

Fig. 2. Size and development of tadpoles. Each symbol represents the average for tadpoles of one species in one pond. Abbreviations as for Fig. 1.

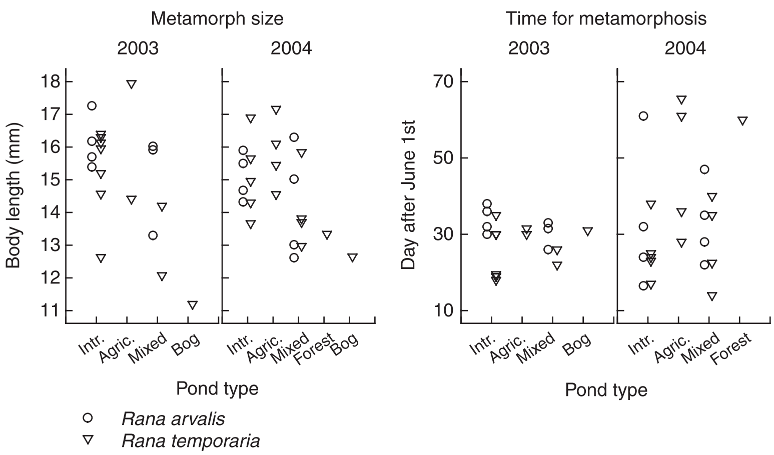

Fig. 3. Metamorph performance. Abbreviations as for Fig. 1.

CHARACTERISTICS OF EXPERIMENTAL AREA PONDS AND REFERENCE PONDS

Ponds in the experimental areas had more algal growth and fewer competitors than reference ponds (Fig. 4; see Table S2 in the supplementary material). There was no significant difference in the number of predators. Experimental ponds scored higher than reference ponds for PC1, i.e. they had a combination of higher pH, conductivity, small size and little tendency to dry up in summer (Fig. 5; see Table S2 in the supplementary material). There was no significant difference for any of the other three PC. The result was the same if only reference ponds with breeding *R. arvalis* were included. Combining all agricultural ponds (those in the experimental areas plus the four reference ponds in agricultural areas), the same differences were found. In addition, this set of agricultural ponds had fewer predators (Fig. 4) and scored higher for PC3 (Fig. 5), i.e. they had higher concentrations of nitrite and nitrate ions than non-agricultural ponds.

TADPOLE PERFORMANCE IN RELATION TO POND CHARACTERS

There were few significant correlations between larval performance and biotic or abiotic characteristics (see Appendix S3 in the supplementary material). Most notable were positive correlations of pH and conductivity with *R. temporaria* survival and development (relative leg length or metamorph performance; *P*-values $< 0{\cdot}005$) and a negative correlation of number of competitors with *R. arvalis* metamorph size ($P < 0{\cdot}001$). All other *P*-values were $> 0{\cdot}01$ and, in light of the possibility of mass significance, they were not considered further. It is difficult to draw any strong conclusions from these correlations. The scatterplots and tests (see Appendix S3 in the supplementary material) are mainly accounted for as a general overview of the data. Of interest is the fact that the scatterplots do not suggest any threshold levels in the present range of biotic and abiotic variables (see Appendix S3 in the supplementary material).

To reduce the tests to a smaller number, with more obvious biological significance, we also tested the effect of the measured biotic and abiotic variables on tadpole performance, with MANCOVAS and ANCOVAS where related variables were combined into one test (Table 4). Among the other independent variables, pond type (experimental area vs. reference pond) was also included.

Table 4. Sets of variables used for MANCOVA tests. All tests were run separately for *R. arvalis*- and *R. temporaria*-dependent variables. Tests involving metamorph performance (numbers 4 and 5) only had one dependent variable and so were ANCOVAS, the other MANCOVAS. The total number of tests was 5 (1–5) × 2 (A and B) × 2 (*R.a.*, *R.t*) = 20

Dependent variable sets	
1	Survival index for free-living tadpoles. Survival for caged tadpoles.
2	Size of free-living tadpoles. Size of tadpoles in cages
3	Relative leg length of free-living tadpoles. Relative leg length caged tadpoles
4	Metamorph size
5	Time of metamorphosis
Independent variable sets	
A	Pond type (experimental area or reference pond). Competitor index. Predator index. Algae weight. Density of spawn
B	Pond type (experimental area or reference pond). PC1. PC2. PC3. PC4

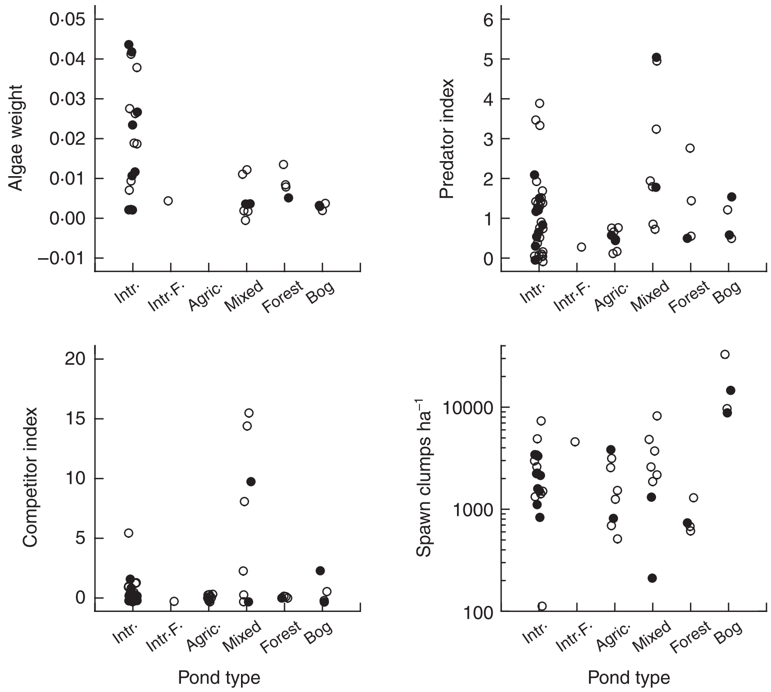

Fig. 4. Biotic characteristics of study ponds. Ponds with data from both years are entered twice. Ponds and years that have been designated outliers with respect to tadpole performance (Table 3) have filled symbols. Intr., introduction; Agric., agricultural.; Intr. F(rog), the introduction pond (Hars1) where spawn of *R. temporaria* was found.

The test thus addressed the question of whether there is any effect of biotic and abiotic variables, in addition to any effects as a result of pond category. The pond type variable included indirect biotic and abiotic effects, i.e. if all agricultural ponds were of 'poor' quality and tadpoles performed poorly, this would not show as a biotic or abiotic effect in this test (although it has been accounted for earlier). Only two of these tests demonstrated any significant effects (non-Bonferroni corrected $P < 0{\cdot}05$). There was a negative effect of the number of competitors on *R. arvalis* metamorph size (ANCOVA, d.f. = 1, $F = 6{\cdot}01$, $P = 0{\cdot}050$) and a positive effect of PC1 on *R. temporaria* metamorph size (ANCOVA, d.f. = 1, $F = 10{\cdot}6$, P = 0·005. A total of 20 different tests (Table 4) was performed so these effects must remain in doubt, considering the possibility of mass significance.

Discussion

DISTRIBUTION IN AGRICULTURAL HABITATS

Tadpoles of both species survived well in most of the ponds in the experimental areas where previously both species were almost (*R. temporaria*) or entirely (*R. arvalis*) absent. The average performance was usually as good as, or even better than, in ponds where native populations are found.

The fact that the reference ponds were not a random sample, but deliberately chosen to represent different types of ponds where the species are found, should increase the variance in reference pond character. This means that these tests are conservative. Had a difference been found, this would have been more striking than would have been the case if the ponds were a truly random sample. However, we emphasize that differences were not found. This is not based on the lack of difference in

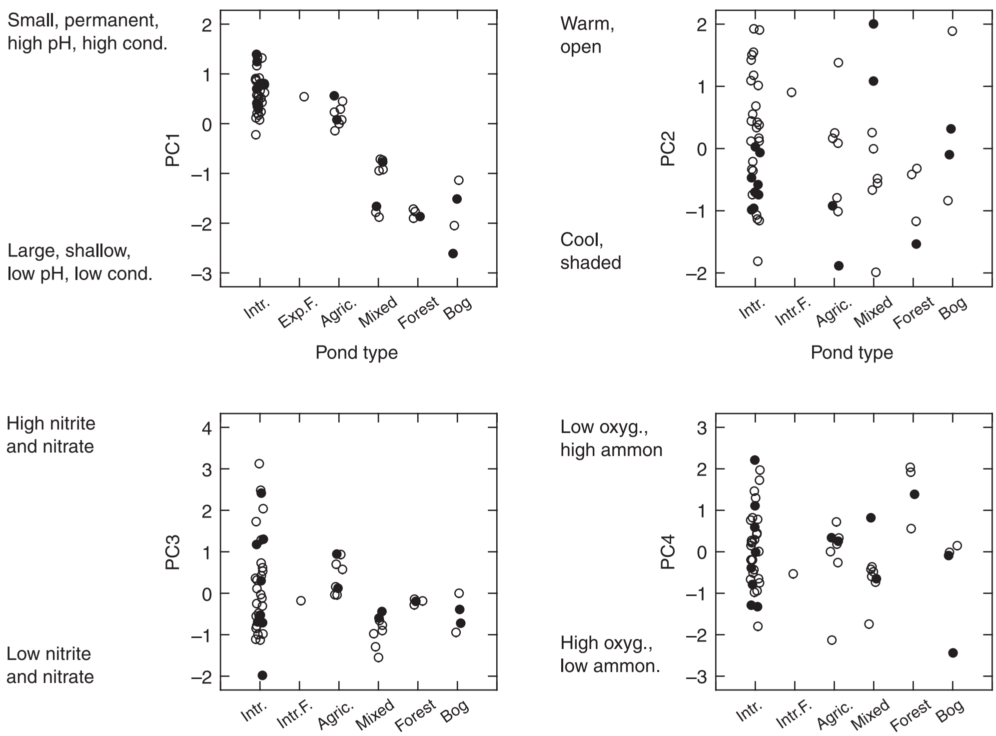

Fig. 5. Abiotic characteristics of study ponds. An interpretation of the principal components is given to the left of each figure (see Appendix S2 in the supplementary material). Cond., conductivity; Oxyg., oxygen saturation; Ammon., ammonium

the tests but on the fact that the range of tadpole performance values was on the whole well within the range of those encountered in ponds with resident frog populations (Figs 1–3). There is one exception to this. Although not significant (which may be because of the low power of the test, see above), extremely poor survival of free-living tadpoles, possibly zero, was more prevalent in experimental than in reference ponds. Some of the ponds in the experimental area seemed to be poorly suited for frog recruitment. This means that the actual density of potential frog ponds may be lower than apparent in these study areas. However, in all the experimental areas there was a number of ponds where both of these species should be able to reproduce successfully.

There is little explicit information on the habitat selection of the two species. *Rana arvalis* mainly favours moorland and pasture (Dierking-Westphal 1987; Podloucky 1987; Kutenkov & Panarin 1995; Fog *et al.* 1997) whereas *R. temporaria* is reported to inhabit a wider range of habitats (Kutenkov & Panarin 1995; Fog *et al*. 1997). Although the occurrence of the latter species in farmland is sometimes mentioned, intensive agriculture is considered a threat (Cooke 1972; Reh & Seitz 1990; Lutz 1992). In a telemetry study, Tramontano (1997) found that *R. temporaria* breeding in an agricultural landscape passed through arable land on their post-breeding migration but settled in patches of other habitats. Overall, *R. temporaria* is occasionally reported in agricultural areas but *R. arvalis* never so (Loman 2005).

In the Scania study area, some other frog species are more tolerant to agricultural land use than the two *Rana* species considered here. One of the rarest species, the spadefoot toad *Pelobates fuscus* Laurenti, has a substantial part of its distribution in intensive agricultural landscapes (Gislén & Kauri 1959; Nyström *et al*. 2002; B. Lardner & J. Loman, personal observation). This is also true for the locally (in the south-western part of the province) common edible frog *Rana esculenta* L. (Gislén & Kauri 1959; J. Loman, personal observation). One of the most common species, the common toad *B. bufo*, is little studied in this area but seems to be fairly abundant in intensive agricultural landscapes (Gislén & Kauri 1959; B. Lardner & J. Loman, personal observation).

DIFFERENCES AMONG PONDS AND EFFECTS ON FROGS

There were few general differences between the ponds in the experimental areas and the reference ponds. In some respect, the ponds in the experimental areas actually appeared more suitable; they had higher pH and conductivity, which may be beneficial for the survival and development of *R. temporaria* tadpoles. There were more epiphytic algae and fewer competing snails, which seemed to be related to large *R. arvalis* metamorphs. Note that the measurements of algae were performed on caged test surfaces; the high weight of algae was thus not a result of few snails.

There were strong numeric correlations (although the significance is unclear because of the large number of tests) between algae weight and tadpole size that may be related to the large size of tadpoles in some agricultural ponds. Indeed, in a previous study the largest *R. temporaria* tadpoles were found in agricultural landscapes (ponds HL3, HL6, HL7, SK6, SK7 in fig. 7 of Loman 2002b). However, a specific analysis of the causal relation between landscape, abiotic factors, algae and tadpole size is outside the scope of this study so the relationship cannot be confirmed.

A negative effect of competitors was suggested. If real, this circumstance will favour tadpoles in the experimental areas where the number of competitors (snails) was invariably low. However, this was only evident as an effect on *R. arvalis* metamorph size; there was no indication of an effect on tadpole size or survival of either species. This effect remains to be confirmed. Other studies suggest complex relations between tadpoles and snails; although they both feed on periphytic algae, a positive effect of snails on tadpole growth, possibly because of indirect enhancement of food availability, has been reported (Brönmark, Rundle & Erlandsson 1991).

The most notable difference between ponds in experimental areas and reference ponds was for PC1, indicating that ponds in the experimental area were small, permanent, basic and had a high conductivity. All these characteristics are linked to their history as marl pits. However, there is nothing suggesting these values *per se* negatively affected tadpole performance, because with the present range of values no significant effect of the biotic and abiotic factors was found, nor were there indications of threshold values. Also, none of the ponds that were negative outliers with respect to tadpole performance had a particularly high value for PC1. Of the component variables, high pH and conductivity (thus high PC1) were associated with experimental ponds and had, if any, a positive correlation with *R. temporaria* survival and metamorph size and time. The lack of any corresponding correlation for R. arvalis could be related to the fact that this species may be more tolerant of acid conditions than *R. temporaria* (Leuven *et al*. 1986; Andrén *et al*. 1988).

PC3 had a high value for ponds in agricultural land. This is not surprising as it indicates high nitrate and nitrite concentrations, typical for heavily fertilized agricultural areas (Bishop *et al*. 1999; Honisch, Hellmeier & Weiss 2002; this study). Several studies have investigated the sensitivity of amphibians to nitrate and nitrite. Although the toxicity is beyond doubt, the harmful effects in the field are less clear. It seems that harmful effects are obvious only at levels encountered in extreme landscapes or by more sensitive species (Marco, Quilchano & Blaustein 1999; Schuytema & Nebeker 1999; Laposata & Dunson 2000; Johansson, Räsänen & Merilä 2001; de Solla *et al*. 2002; Pellet 2002; Ortiz *et al*. 2004). Oldham *et al*. (1997) found that adult frogs suffered acute ill effects from fertilizer spread onto them. In our study, the nitrite and nitrate levels encountered did not seem to affect tadpole performance. The only exception was pond Borg3, where there was poor survival of *R. arvalis* and a high value for PC3, possibly related to the provision of food for ducklings in this pond. Schuytema & Nebeker (1999) found that ammonium nitrate and sodium nitrate concentrations in excess of 100 mg/L caused approximately 50% mortality in tadpoles of *Pseudacris regilla* but none below approximately 10 mg/L. In our study the highest nitrate concentrations measured were approximately 10 mg/L in two ponds (Borg2 and Borg3) but concentrations did not exceed 2 mg/L in other ponds (see Appendix S3 in the supplementary material). Thus, adverse effects of nitrate would probably not show up in our study areas.

Pesticides have been shown to harm frogs, directly or indirectly, in agricultural habitats (Quellet *et al*. 1997; Bishop *et al*. 1999; Gutleb *et al*. 1999; Davidson, Shaffer & Jennings 2001; Sparling & Fellers 2001; Relyea 2003; Bridges 2004; Fellers *et al*. 2004; Storrs 2004). In the present case we do not think this was important. In our experimental areas, tadpoles generally did not perform any worse than in the reference areas and poor performance was not specific to any of the experimental areas but scattered in single ponds. In no pond was there a total failure both for free-living and caged tadpoles, such as would be expected had there been a local catastrophic release of pesticides.

Ponds in agricultural areas are often stocked with fish or crayfish, which can negatively affect frog populations (Glandt 1983; Brönmark & Edenhamnn 1994; Axelsson *et al*. 1997; Hecnar & M'Closkey 1997; Laurila & Aho 1997). Some fish, notably *Carassius carassius* L. and *Pungitius pungitius* L., often appear without human intervention. Fish were not specifically sampled in our study but *C. carassius* was found in the scraper net samples of one of the study ponds in an experimental area (Rem10). The fact that it was not found in any of the others suggests it was not present in high numbers there.

None of the characteristics associated with agricultural land was consistently detrimental to tadpole performance in Scania. This is clear from the scattered distribution of tadpole performance (filled symbols) on all panels of Figs 4 and 5. How does this fit with the observations that there were several poor performance ponds in these areas? There are a number of explanatory factors, for example the dumping of plant material such as waste sugarbeet *Beta vulgaris* L. possibly resulting in a high oxygen demand, or intensive feeding of ducklings, affecting nitrogen load. Not all of these episodes affected our abiotic measurements and in some cases there was no obvious harmful effect on tadpoles, possibly because the harmful effect was of short duration or appeared too late. For example, in pond Sven11, a quantity of sugarbeet was dumped in the pond and was followed by extreme low values for dissolved O_2 in early May (2% saturation), although by late May the pond was recovering (16% saturation). However, all eggs introduced in the pond in mid-April died before hatching. In one of the ponds with duck feeders (Borg5), dissolved O_2 dropped from 48% saturation in early May to 10% in late May when (unsuccessful) netting for tadpoles took place. In contrast, caged tadpoles in Borg5 had 67% survival (both species) and were of average size. Only in ponds Sven15 and Harl8 was there no obvious reason for the poor tadpole performance. Overall, aspects of water chemistry often seem to have had only moderate impact on the amphibians (Laan & Verboom 1990; Hecnar & M'Closkey 1996).

In conclusion, no specific factor was found to be of overriding importance for tadpole survival in this area. However, some factors were found to negatively affect survival. In particular, dumping of biological material will reduce levels of dissolved O_2 and will raise nitrate levels with detrimental effects on amphibians. This may be a typical problem in agricultural areas.

Many studies report an impoverished frog fauna in agricultural landscapes (Kolozsvary & Swihart 1999; Bishop *et al*. 1999; Guerry & Hunter 2002), although an exception is provided by Knutson *et al*. (1999). What are the reasons for this general pattern? Some studies have indeed demonstrated poorer performance of tadpoles in ponds in agricultural areas. Laposata & Dunson (2000) reported poor hatching success for *Rana sylvatica* LeConte eggs and poor survival of tadpoles that had been experimentally raised *in situ* in highly nutrient rich ponds. de Solla *et al*. (2002) observed similar effects on eggs and tadpoles of *Rana aurora* Baird & Givard in an agricultural area in British Columbia. However, no specific biotic or abiotic factors were shown to be responsible.

Rather than focusing on pond quality, it may be that the suitability of the agricultural landscape for frogs is more dependent on patterns related directly to the landscape. The quality of the terrestrial habitat may be an important consideration (Porej, Micacchion & Hetherington 2004), for example landscape fragmentation *per se* may limit the distribution of frog populations (Halley, Oldham & Arntzen 1996; Sjögren-Gulve 1998; Kolozsvary & Swihart 1999; Lehtinen, Galatowitsch & Tester 1999; Pope, Fahrig & Merriam 2000; Semlitsch 2000; Marsh & Trenham 2001; Vos *et al*. 2001). We suggest that metapopulation aspects, including access to different terrestrial key habitats, may be responsible for the lack of *R. arvalis* and *R. temporaria* in the agricultural landscapes of western Scania.

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Supplementary material

The following supplementary material is available as part of the online article (full text) from http://w.w.w.blackwell-synergy.com.

Appendix S1. Additional information on methods

Appendix S2. Results of principal component analysis and correlations among physical and biotic effect variables

Appendix S3. Overview of environmental and tadpole performance variables and their raw correlations

Fig. S1. Map of Scania, south Sweden, with reference and experimental areas

Table S1. Tests for differences between performance of tadpoles in introduction ponds and reference ponds

Table S2. Tests for differences in characteristics of experimental and reference ponds