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A documented amphibian decline over 40 years: Possible causes and implications for species recovery

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ABSTRACT

Amphibians are declining worldwide, but lack of long-term regional data makes identifying possible causes difficult, hindering conservation efforts. We evaluated whether habitat destruction, terrestrial habitat adjacent to ponds and the physico-chemical characteristics of ponds could explain the regional and local decline of the spadefoot toad (*Pelobates fuscus*) in Sweden.

Analyses of aerial photos and field observations revealed that out of all of the known calling sites of the species since 1959, 26% did not exist in 2000. The road traffic intensity adjacent to existing ponds indicated that in 1997–2003 it was higher near ponds where calling males had disappeared ($N = 240$) compared to sites where calling males were present ($N = 84$). The soil-type adjacent to ponds with calling males was more sandy than at ponds where calling males had disappeared (instead dominated by till). By including road traffic intensity and proportion of sandy soils adjacent to ponds, a logistic regression model correctly classified 82% of the ponds into their correct category.

Of 36 ponds investigated in 2004, we found evidence of successful reproduction (tadpoles) in 53%. Unsuccessful reproduction seemed to be associated with eutrophication and low coverage of submerged macrophytes. In an area with low road traffic intensity and sandy soils, restoration of ponds started in 1996, and the number of calling males increased from a maximum of 77 in 1993–1996 to 146 in 2006. Our results indicate that habitat destruction has likely contributed to the regional decline of *P. fuscus*, but also that local factors such as soil type, traffic intensity and reproductive failure may also help explain the decline of the species.

1. Introduction

Amphibian populations have been declining globally in recent years and unless these declines are understood and reversed, hundreds of species may become extinct over the next few decades (e.g. Stuart et al., 2004). Amphibian population dynamics are often influenced by processes at different scales and in order to understand the factors influencing amphibian population size it is important that the spatial and temporal scale studied is relevant to the target species (Skelly et al., 2003; Storfer, 2003). Thus, studies of declining amphibians should examine the effects of multiple factors that potentially affect reproductive success and survival in different habitats. Only when the factors regulating populations of

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threatened species are known can we understand why they decline, knowledge that is crucial if conservation efforts are to be successful.

For amphibians it seems that habitat loss and over-exploitation of adults by humans for food are important factors behind most declines, but other unknown processes threaten 48% of rapidly declining species (Stuart et al., 2004). A major concern is that in many cases there is almost no evidence of recovery of amphibian populations and no known method for saving mysteriously declining species (Stokstad, 2004). Identification of the mechanisms causing declines should be given high priority and the knowledge gained should be used in restoration projects. Because the major reason for the amphibian decline in Europe seems to be habitat loss, habitat restoration should therefore be an important counter measure (Beebee, 1997; Fog, 1997; Stumpel and van der Voet, 1998). However, for restoration projects to be successful and to optimize conservation efforts it is important to identify particular areas and habitats where restoration is expected to give the best results.

The spadefoot toad *Pelobates fuscus* is widely distributed in Europe, but has declined dramatically in numbers within its northern distribution range, especially in Denmark (Fog, 1997; Fog et al., 1997) and Sweden (Berglund, 1998; Nyström et al., 2002). In 1945–1990 it disappeared from 98% of known breeding ponds in Denmark (Fog et al., 1997). The distribution of *P. fuscus* in Sweden has been recorded since 1959. Of 427 ponds where it was present in the 1960s and 1970s, only 60 had calling males in 1993–1996 (Berglund, 1998). Thus, in the 1960s and 1970s it was estimated that the total number of calling males in Sweden was about 100,000, whereas the total number of calling males in 1993–1996 was between 400 and 850 (Berglund, 1998). Several causes for the decline have been suggested, including the destruction of breeding habitats, habitat fragmentation and increased road traffic load (Corbett, 1989; Gasc et al., 1997; Hels and Buchwald, 2001), but other changes to breeding habitats and/or surrounding terrestrial habitats also are likely to be contributing factors, since *P. fuscus* has disappeared from several ponds in relatively pristine areas (Berglund, 1998). For example, it has been shown recently that *P. fuscus* is present only in nutrient rich, sun-exposed permanent ponds without predatory fish and with low abundances of exotic crayfish (Nyström et al., 2002). Moreover, even if ponds are suitable for the species it also appears to be in decline in areas without sandy soils. Since *P. fuscus* hibernates in the soil and is often associated with agricultural landscapes, it may suffer heavy mortality during the terrestrial stage due to intense farming. This may be because *P. fuscus* cannot burrow so deeply in clay and moraine soils and hence can be more exposed to ploughing than in sandy soils (Berglund, 1998).

The factors affecting population size in *P. fuscus* are less well known, but the work of Hels (2002) suggests that population size could be determined by density dependent survival during the larval stage. Also, heavy mortality in the terrestrial stage (e.g. road kills) may reduce the number of eggs laid below a critical point where all eggs and juveniles are consumed by predators. The low dispersal rates of adults between ponds may cause local extinction in the long-term as a result of reproductive failure since many populations are dependent on immigration by froglets from other populations in order to persist.

Thus, apart from habitat destruction and increased adult mortality during the terrestrial stage, *P. fuscus* may also have limited reproductive success in its present breeding ponds (e.g. Nielsen and Dige, 1995), potentially explaining declines even in areas with low traffic intensity and sandy soils. An important change in agricultural practices, apart from farming methods is the increasing use of chemical fertilizers and pesticides. Chemical fertilizers or manure from livestock along with pesticides may affect survival and growth during the larval stages of many amphibians (Marco et al., 1999; Rouse et al., 1999; Johansson et al., 2001; Ortiz et al., 2004; Teplitsky et al., 2005). Successful reproduction of *P. fuscus* has been confirmed in only about 50% of eutrophic ponds in some parts of Denmark (Hansen, 2002). Suggesting that changes in water chemistry associated with agriculture and livestock farming may have influenced the reproductive success and local population densities of *P. fuscus* in some areas.

The aim of this study was to increase our knowledge of why *P. fuscus* has declined in numbers in Sweden and so increase our ability to make appropriate conservation efforts. Specifically, we were interested in: (1) estimating the percentage of breeding ponds that have been lost as a result of habitat destruction, (2) determining whether land-use, soil-type and road traffic load differ around sites with and without calling males, (3) determining the reproductive success of *P. fuscus* and relating it to physico-chemical characteristics of the ponds and their adjacent terrestrial environment, and (4) evaluating the results of restoration efforts in a relative pristine area (i.e. with a traffic load of less than 10 vehicles per day, Nyström et al., 2002) where the number of calling males has been monitored almost every year since 1993, and where restorations of ponds started in 1996.

2. Methods

2.1. Natural history

P. fuscus inhabits in the warmer part of Sweden and its distribution range is therefore restricted to Scania in the south of the country (Gasc et al., 1997; Nyström et al., 2002, Fig. 1). Reproduction starts in the beginning of April and ends in May. A female lays about 2000 eggs in a string, which is often wrapped around submerged macrophytes at a depth of 10–40 cm (Strijbosch, 1979). Embryos hatch after about seven days, and newly hatched larvae need a temperature above 15 °C to develop normally and subsequently to metamorphose (Jensen, 1992). The larval stage is long compared to that of other amphibians in Sweden and lasts for 3–4 months depending on water temperature and food abundance. Adults spend most of their terrestrial life close to the breeding pond and rarely disperse more than 500 m from it (Nöllert, 1990; Nielsen and Dige, 1995; Hels, 2002).

2.2. Habitat destruction, soil-type and traffic intensity

We quantified the frequency of habitat loss for *P. fuscus* using data on calling males from all 453 breeding sites known in Sweden between 1959 and 2003 (Fig. 1). The complete data set was obtained from the County Administrative Board of Scania and all data are based on investigations made by Berglund (1998). GIS (Arc GIS 8.3) was used to investigate how many of the known calling sites had been destroyed by determining their presence or absence on 55 aerial photos (orthophotos, i.e. geometrically correct aerial photos) in the year 2000. The photos were taken between May and September during the period when *P. fuscus* starts breeding and larval development occurs. Each photo covered an area of 5×5 km^2. The orthophotos were overlain in GIS by a point layer showing the 453 known calling sites for *P. fuscus*. This method made it possible to check visually whether a site was present or not on the orthophoto. We also noted if houses overbuilt the site and refer to this condition as lost due to urbanization. In most cases we could categorize a site as destroyed or present with confidence, but in some cases

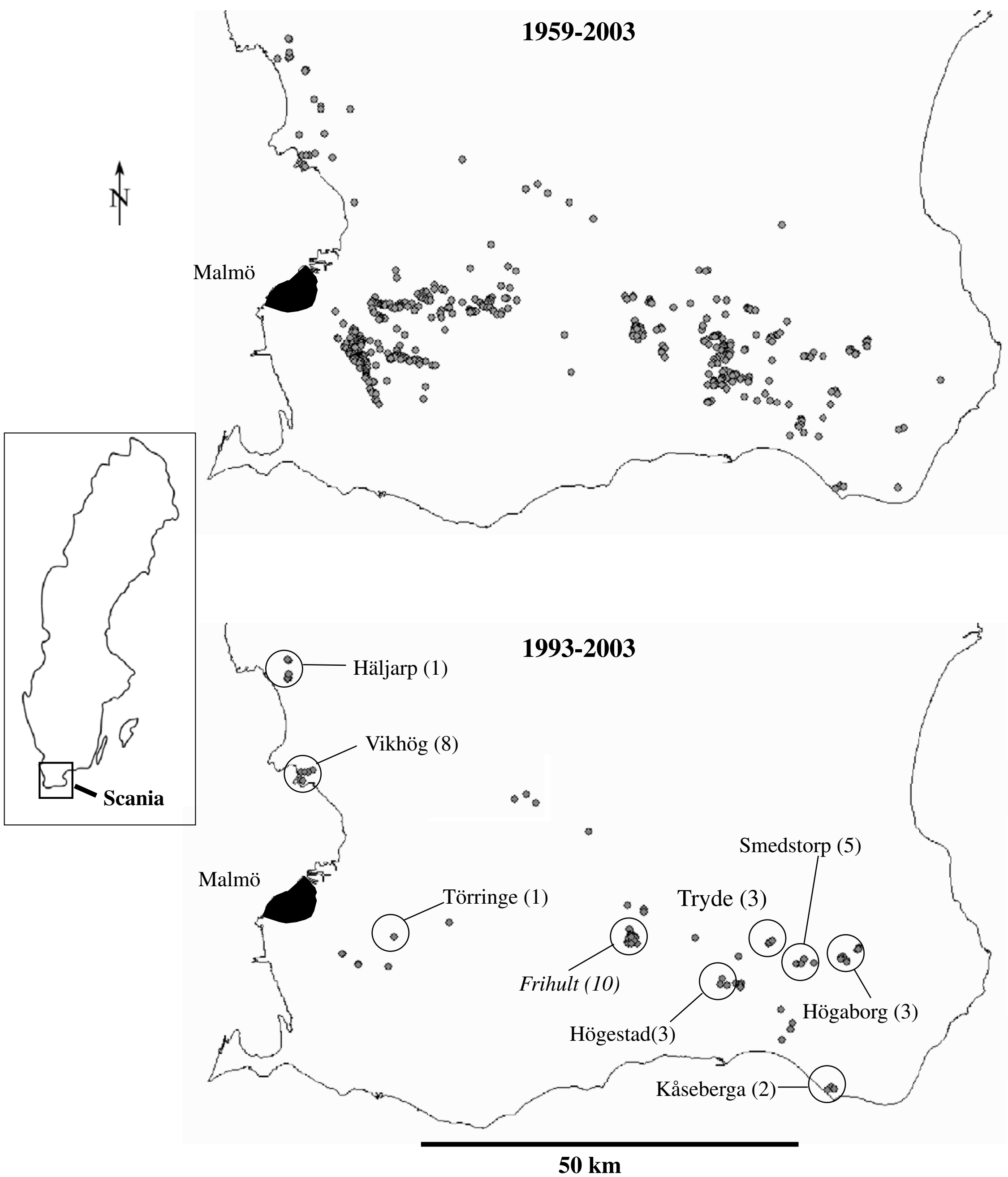

Fig. 1 – All known sites with calling males of *Pelobates fuscus* in Sweden (Scania) between 1959 and 2003 and those remaining between 1993 and 2003. The nine areas surveyed for evidence of reproduction in 2004 are encircled (with number of sites investigated with observed egg-strings and/or at least five calling males in parenthesis). Responses of a *P. fuscus* population (number of calling males) to pond restorations were made in the Frihult area (italic) in 1993–2005. Location of the largest city in Scania (Malmö) is shown. The County Administrative Board of Scania provided distribution data for *P. fuscus*, These data are based on information provided by Berglund, Pröjts and the authors (see also Berglund, 1998).

(43 sites), we were uncertain. For example, if a site was a temporary pond and the aerial photo was taken late in the breeding season, the pond could still be an existing site. If the site was located within a forest patch it was also unclear if the site existed or not. Therefore, in March and April 2004 the uncertain sites were revisited to confirm their existence or absence. Nevertheless, we remained uncertain about 16 sites, which were excluded from all analyses. Therefore, in total we used 437 sites.

To be able to compare soil-types near ponds with and without calling males, we assumed that *P. fuscus* is found typically within a 500 m radius of its breeding pond (Nielsen and Dige, 1995; Fog et al., 1997; Hels, 2002; Nyström et al., 2002). We based our selection of sites and the categories "males absent" or "males present" on the inventory data provided by the County Administrative Board of Scania. Since all remaining breeding sites for *P. fuscus* were investigated in 1993–1996 (Berglund, 1998), we assumed that if calling males were absent during those years, the species had disappeared. In addition, eleven new calling sites were found after this major inventory and the County Administrative Board of Scania provided the coordinates for them. In total 324 sites were physically still present, 84 with calling males and 240 from which *P. fuscus* had disappeared. The 324 sites were analyzed in terms of proportions of cultivated fields and soil-type within a 500 m radius circle around each site. For this purpose digital land-use and soil maps were used. The original soil map was adjusted so that the number of soil-types was reduced from 57 to 13 based on grain size. The dominant soil-types: till, clayey till, clay, sand, and silt, were used in the final analysis. In GIS a point layer with buffer zones of 500 m radius around each site was created. The buffer zone layer was then combined with the land-use map, and using a clip-out operation the land-use within the 500 m radius around each site was extracted. The same operation was used to extract the soil-types around sites. Within each buffer zone the proportion of different land-uses (e.g. cultivated fields) and soil-types was calculated.

Road traffic intensity near ponds was estimated using the program EnviMan AQ Emissioner, 3.0.8 (Opsis AB, Sweden). Data used for the estimation of traffic intensity were provided by the Swedish National Road administration for 1997–2003. The program calculates the total number of kilometers travelled by vehicles on all public roads within a chosen buffer zone (in this case a square of 1×1 km, with the site of interest in the middle).

We tested whether the proportion of land-used for agriculture, the proportion of sandy soils and traffic intensity differed around sites where *P. fuscus* are still present compared to sites where the species has disappeared using stepwise logistic regression using the backward elimination procedure (Quinn and Keoughm, 2002). The initial model included all main effects (proportion of land-used for agriculture, the proportion of sandy soils) and all two-way and three-way interactions. The backward stepwise selection was then conducted testing the significance of the interactions and main effects (SPSS 11.0 for MacIntosh). If the three-way interaction was not significant it was removed from the model and the next analysis included main effects and two-way interactions. The final model included only significant main effects and significant interactions.

2.3. *Reproductive success in relation to habitat quality*

In 2004 we surveyed 36 permanent ponds in Scania. All were known to have calling males of *P. fuscus* in recent years and were free of predatory fish and crayfish (Nyström et al., 2002). Ponds were chosen in nine different areas (Fig. 1) because our intention was to include ponds encompassing the entire distributional range of *P. fuscus* in Sweden in order to assess its reproductive success at both the regional and local scale. The sites were visited in spring (26 April– 4 May) during the peak of the breeding season. The number of calling males was counted at night, but we also searched for egg-strings during the day. Between 6 May and 13 June 2004, we characterized 32 biological, chemical and physical parameters of the 36 ponds as described in Nyström et al. (2002). The chosen parameters included factors expected to affect reproductive success either directly or indirectly such as water chemistry, temperature, macrophyte coverage and land-use close to the pond. In addition, since temperature, oxygen concentration and the development of macrophytes may change significantly during the period of larval development and affect reproductive success in *P. fuscus* (Strijbosch, 1979), these parameters were measured also later in the summer (between 21 June and 22 June, and between 6 July and 8 July). Apart from oxygen concentration and temperature, which were measured in the ponds directly (Nyström et al., 2002), other water quality parameters were analyzed by an accredited laboratory (Lennart Månsson International, Helsingborg, Sweden). Measurements of nitrate, ammonia and chloride were made on an autoanalyser (segmented flow), and other elements by ICP-OES. The detection limit for all elements was 1 μg/l.

A preferable way of estimating reproductive success in a pond is to capture all the metamorphs and measure their size (e.g. Nielsen and Dige, 1995), however, we were unable to do that. Instead, we defined reproductive success by the presence of larvae in late summer (between 6 July and 4 August), just before metamorphosis normally occurs. We assigned a pond to the category "reproductive failure" if we did not observe any larvae and if we did not catch any larvae using a handnet (diameter 30 cm) deployed every 15 m around the circumference of each pond. If no larvae were caught or observed, the procedure was repeated up to three more times. In our survey we included only ponds in which larvae could be observed or captured effectively. Moreover, we only included ponds with at least five calling males to ensure that at least one female was present, because of the skewed sex ratio (1.7 males: 1 female, Hels, 2002), or ponds where egg-strings were observed. Therefore, of the 52 ponds surveyed for larvae only 36 were included in the final analysis.

To test whether ponds with and without the presence of larvae differed with respect to their biological and physicochemical characteristics, and to identify the factors that were most important for successful reproduction or not, we used a combination of principal components analysis (PCA) and stepwise discriminant analysis (e.g. Hecnar and M'closkey, 1996). Since we had environmental variables that were autocorrelated, and because the number of ponds were few relative to the number of environmental variables, we reduced the 32 original variables into fewer uncorrelated new variables using PCA (discussed in Stumpel and van der Voet, 1998). We used a correlation matrix with varimax rotation and axes with eigenvalues >1 were retained. We based our analysis and interpretation chiefly on the recommendations given by Dillon and Goldstein (1984). Interpretations were therefore based on those variables loading highest on a given factor. Environmental variables with an absolute loading of $\geqslant 0.35$ (corresponding to a significance value of $P = 0.05$ for the correlation coefficient when $N = 32$) on a given axis were considered to be important. The derived scores for the retained principal components were entered in the discriminant function analysis (SPSS 11.0 for MacIntosh) with the validity of the discriminant function being estimated using jackknife classification. This involved allocating each pond to its closest group (in this case ponds with or without reproduction) without using that pond to help determine the group centre (Manly, 1994). To meet the assumptions of the analyses, data in proportions were arc sine transformed prior to analysis and other data (except pH) were natural log-transformed.

2.4. Effects of habitat restoration on the number of calling males

In a remote area with low road traffic intensity (fewer than 10 vehicles per day, Nyström et al., 2002, Frihult, Fig. 1), we assessed colonization and responses in terms of number of calling males of an isolated metapopulation of *P. fuscus* to increased number of ponds, and to pond restoration. The area contained about 25% of all known calling sites for *P. fuscus* in Sweden and was dominated by sandy soils. However, the characteristics of the terrestrial environment in which the 45 ponds were set differed. Of these ponds 89% were located in pasture, 36% in agricultural land, and 27% in forest. Thus, several ponds had surroundings consisting of a mixture of pasture, agriculture and forest. In spring (2004) the median depth of the ponds was 1 m (range 0.1–1.75), and the median area of the ponds was 500 m^2 (range 1–4000). Some of the 45 ponds were temporary (6), and others contained predatory fish (5) and were not used by *P. fuscus*. (Nyström et al., 2002). All ponds were within the dispersal range of *P. fuscus*, however, as they were all within an area of 1.4×1.3 km. Seven species of amphibians bred in the area: *P. fuscus*, *Hyla arborea*, *Bufo bufo*, *Rana temporaria*, *Rana arvalis*, *Triturus cristatus* and *Triturus vulgaris*.

In Frihult, *P. fuscus* was found in 21 ponds before the survey in 1993–1996 (Berglund, 1998), but it has disappeared from at least four ponds that now contain predatory fish and several other ponds have been drained or become unsuitable because of forest plantations (Berglund, 1998). Between 1996 and 2002, 15 ponds were restored (a combination of dredging and removal of shading trees) and four new ponds were created. We and Berglund monitored the *P. fuscus* population in Frihult (Fig. 1) between 1993 and 2006 to assess the population response to pond restoration. In spring every year, the number of calling males was counted during three visits, and the maximum number of calling males in each pond determined. To get a rough estimate of total population size in the area we summarized the maximum number of calling males for all ponds heard during three weeks at the peak of the breeding season in each year. Even though calling males may have moved among ponds during the survey and thus been counted twice, the exchange rate between ponds by adults is often very low (Hels, 2002) and should not have influenced our estimate of the numbers of calling males, significantly between years. In 2004, all ponds in Frihult with calling males of *P. fuscus* (22 ponds of which 14 had been restored or were new) were also surveyed for successful reproduction (as described above in methods). To obtain information on colonization by amphibians in general to habitat restorations we also searched for eggs (*B. bufo*, *R. temporaria* and *R. arvalis*), calling males (*H. arborea*) and larvae (*T. cristatus* and *T. vulgaris*) of other species known to be present in the area.

3. Results

3.1. Habitat destruction

Analysis of aerial photos in 2000 and field observations in 2004 showed that 26% of the 437 ponds known to have calling males of *P. fuscus* sometime in 1959–2003 did not exist any more. Of these ponds, 13% were classified as lost because of urbanisation, and the remaining non-existing ponds were classified as being lost as a result of draining or successional processes. The largest reduction of sites was seen in the western part of the Scania (in the vicinity of the expanding city of Malmö, Fig. 1) where traffic intensity was comparatively high and clayey till is the predominant soil-type. The reduction was smaller in the eastern part of Scania where in general sandy soils are more common.

3.2. Terrestrial habitat characteristics near ponds with and without calling males

In 2000, the proportion of land-used for agriculture within a radius of 500 m of the ponds was estimated to be 54% (±26 SD) around ponds with calling males ($N = 84$) and 60% (±26 SD) around ponds where calling males have disappeared ($N = 240$). The proportion of sandy soils within this 500 m radius showed that ponds with calling males were located in areas with proportionately more sandy soils (46% ± 28 SD) than were ponds without calling males (14% ± 22 SD, Fig. 2). Instead, till was the dominant soil-type near ponds without calling males (Fig. 2). Between 1997 and 2003 road traffic intensity in terms of vehicles $\times 10^6$ km/year was higher near ponds without calling males (1.4 ± 3.3 SD) than near ponds with calling males (0.4 ± 0.7 SD). However, sites predominantly located on sandy soils were also located in areas with low traffic intensity (Fig. 3). We modelled the presence/absence of calling males against three predictor variables (proportion of land-used for agriculture, proportion of sandy soils and road traffic intensity) in a stepwise backward logistic regression analysis. The final model ($\chi^2 = 109.0$, $P < 0.001$, Nagelkerke $r^2 = 0.41$, $N = 324$) included only the main effects of proportion of sandy soils ($P = 0.001$) and road traffic intensity ($P = 0.043$) and these two variables correctly predicted 82.5% of the sites with or without calling males. This suggests that calling males have disappeared from sites located in areas with a low proportion of sandy soils and high road traffic intensity (Figs. 2 and 3).

3.3. Reproductive success

Presence of late-stage tadpoles was found in 19 of 36 ponds in late summer. The number of calling males at the sites without tadpoles varied between 0 and 70 and between 1 and 120 at sites with tadpoles. Egg-strings were observed in 6 of the ponds with the presence of tadpoles and in 4 ponds where no tadpoles were found (Table 1). Eighty percent of the variation in 32 physical, chemical and biological characteristics measured in the ponds and in their adjacent terrestrial surroundings (Table 2) were reduced to eight axes using PCA. The scores for axes two and three differed significantly between ponds with and without the presence of tadpoles (Table 3, Fig. 4), but scores for the other axes did not differ, significantly ($P > 0.12$ in all cases). Axis one was correlated with two terrestrial land-use variables (forest and pasture) and six chemical pond water variables; axis two was correlated with several chemical variables, including some associated with water quality (eutrophication). Axis three was correlated with coverage of submerged macrophytes and the percentage of shallow habitat at a pond's edge (Table 3, Fig. 4).

To estimate how well the environmental variables could predict the presence of tadpoles, the scores for the eight axes were included in a stepwise discriminant function analysis. The analysis successfully classified 77.8% of the 36 ponds in their correct category (Wilks $\lambda = 0.67$, $P < 0.001$). Classification success was higher for ponds without tadpoles (88.2%) than for ponds with tadpoles (68.4%). Eight of the 36 ponds were misclassified. A jackknife method also classified 77.8% of the ponds correctly. The stepwise discriminant function analysis was able to separate ponds into their correct category based on the scores of the third and second PCA axes. Thus, most ponds without tadpoles had lower coverage of submerged macrophytes and a lower percentage of shallow habitat in the littoral zone (PCA axis 3, Tables 2 and 3) than ponds with tadpoles. Moreover, sites without tadpoles present generally were more eutrophic, had lower oxygen concentrations and higher concentrations of metals such as iron and manganese (PCA axis 2, Tables 2 and 3).

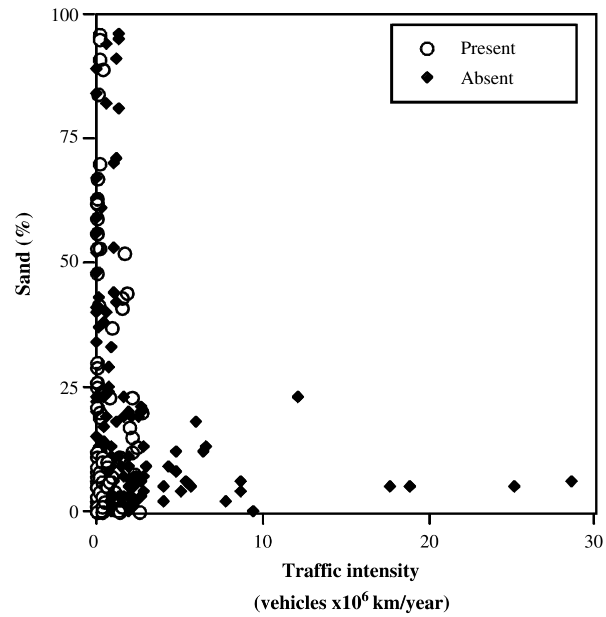

Fig. 3 – Relationship between traffic intensity (measurements made between 1997 and 2003) and percentage of sandy soils within 500 m for ponds with calling males of *Pelobates fuscus* still present (N = 84, open circles) or absent (N = 240, filled diamonds) during surveys between 1993 and 2003.

3.4. Responses of the number of calling males to habitat restoration

In the vicinity of Frihult (Fig. 1) the total number of calling males reported per year before restoration and the construction of new ponds varied between 35 and 77. However, since 1996 when restoration started, the number of calling males increased and in 2006, at least 146 calling males were recorded in 22 different ponds (Fig. 5). In 2004 when reproductive success was investigated in all ponds with calling males (22 ponds), tadpoles were found in five ponds only and all these ponds had been restored (Fig. 1, Table 4).

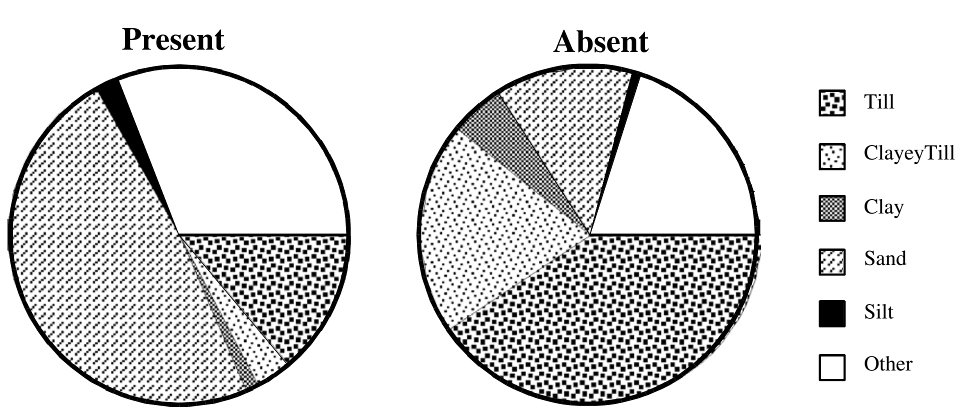

Fig. 2 – Percentages of different soil-types within a radius of 500 m for ponds with calling males of *Pelobates fuscus* still present (N = 84) or absent (N = 240) during surveys between 1993 and 2003.

Table 1 – Ponds with or without tadpoles of *Pelobates fuscus* observed or netted during the survey in 2004

With tadpoles			Without tadpoles		
Pond	Calling males	Egg-strings	Pond	Calling males	Egg-strings
86–94 (Kåseberga)	7	–	86–92 (Kåseberga)	30	+
86–56 (Högestad)	35[a]	–	86–50 (Högestad)	20[a]	–
70–11 (Smedstorp)	50[a]	+	86–62 (Högestad)	70[a]	–
S. Mattiasdammen (Smedstorp)[c]	40[a]	+	Videdammen (Högaborg)[b]	0	+
Mattiasdammen (Smedstorp)[c]	60[a]	+	Nygrävda dammen (Högaborg)[b]	1	+
N Mattiasdammen (Smedstorp)[c]	60[a]	+	70–03 (Tryde)	3	+
70–09 (Smedstorp)	100[a]	+	70–06 (Tryde)	12	–
70–19 (Högaborg)	120[a]	–	Åkerdammen (Törringe)[b]	14[a]	–
70–04 (Tryde)	1	+	61–12 (Vikhög)	25	–
61–11 (Vikhög)	30	–	61–24 (Vikhög)	15	–
61–08 (Vikhög)	40	–	61–09 (Vikhög)	6	–
61–07 (Vikhög)	10	–	61–10 (Vikhög)	8	–
61–23 (Vikhög)	15	–	65–69 (Frihult, pond 4)	15	–
82–01 (Häljarp)	100[b]	–	65–28 (Frihult, pond 8)	15	–
65–32 (Frihult, pond 12)	7	–	65–71 (Frihult, pond 10)	5	–
65–36 (Frihult, pond 23)	7	–	65–34 (Frihult, pond 14)	6	–
65–43 (Frihult, pond 24)	10	–	65–48 (Frihult, pond 37)	8[a]	–
65–37 (Frihult, pond 30)	20	–			
65–38 (Frihult, pond 31)	5	–			

The maximum number of calling males heard and observations of egg-strings (+/−) are shown pond numbers and names are given as officially used by the County Administrative Board of Scania. Ponds in italic type were misclassified in a discriminant function analysis using abiotic and biotic characteristics of the ponds.
a Observation by Boris Berglund.
b Observation by Jan Pröjts.
c Unofficial name.

4. Discussion

4.1. Habitat destruction

Urbanization and human activities negatively affect amphibians as a result of the destruction of breeding habitats, fragmentation of populations and fish introductions Andreone et al., 2005; Crochet et al., 2004; Haddad and Prado, 2005; Riley et al., 2005; Cushman, 2006). The landscape in Scania has been transformed from an area dominated by pasture, small-scale farming and numerous wetlands in the 1950s to an urbanized area in the south–west, and also to areas with large-scale agriculture, forest plantations, and as a consequence, comparatively few ponds. By comparing the number of wetlands and ponds on aerial photos from 1940 and 2000 we estimated the percentage of ponds lost in Scania to be about 45% (results not shown). These changes have undoubtedly contributed to the dramatic decline of *P. fuscus* as found by others (Fog et al., 1997; Berglund, 1998). The most important findings of our surveys and analyses are that about 25% of all known breeding habitats of *P. fuscus* known since 1959 were absent in 2000. Within the pelobatidae, habitat loss seems to be the dominant reason for the decline of most species (Stuart et al., 2004). However, despite habitat loss during transformation of the landscape, the decline of *P. fuscus* has been less severe in areas with sandy soils and low road traffic intensity.

4.2. Terrestrial habitat and population size

It is likely that population size in *P. fuscus* is regulated during both aquatic and terrestrial stages, since successful development of the aquatic stages may not result in increased number of adults if the terrestrial habitat is unsuitable, e.g. in areas with compressed soil and a lack of hibernation sites (Jehle et al., 1995). It is also possible that recolonization of ponds by *P. fuscus* is affected by the nature of the terrestrial environment since most of the ponds present now are adjacent to fields, and such agriculturally disturbed areas devoid of cover may disrupt the ability of amphibians to reach new habitat patches (Mazerolle and Desrochers, 2005).

Our results also indicate that road traffic intensity today is higher near ponds from which *P. fuscus* has disappeared. Other studies have also indicated that traffic mortality can have a significant effect on the local density and distribution of anurans (Fahrig et al., 1995; Vos and Chardon, 1998; Hels and Buchwald, 2001) and urodeles (Marsh et al., 2004). For *P. fuscus* in Denmark, it was estimated that 10% of the adult population was killed annually at a traffic load of 3200 vehicles/day, (Hels and Buchwald, 2001). We also found that most sites with *P. fuscus* were in areas with sandy soils, where traffic intensity was generally lower. How traffic and soil-type operate and interact to reduce frog numbers in Scania is not known, but the restoration project in Frihult indicates that *P. fuscus* may respond positively to habitat restoration at least in areas with both low traffic intensity and sandy soils. Further, restoration of ponds in such areas therefore may be of great importance for the conservation of *P. fuscus* most ponds within its current distribution range in southern Sweden (i.e. within 500 m of a natal pond) are unsuitable because of the presence of predatory fish, shading trees, or their temporary nature (Nyström et al., 2002). However, in order to maximize restoration success, future studies of *P. fuscus* need to evaluate the reproductive potential of different habitats.

Table 2 – Component loadings of 32 environmental variables measured in 36 ponds with *Pelobates fuscus* in 2004, and eigenvalues and the percentage variance explained by the first three (out of eight retained) principal component axes

Source	PC1	PC2	PC3
Variance explained (%)	19.08	13.98	11.57
Eigenvalues	7.58	5.92	3.17
Agriculture	**0.81**	−0.16	0.00
Pasture	**−0.81**	0.18	0.00
Forest	0.00	0.00	−0.16
Maximum pond depth	0.12	−0.14	−0.31
Pond area	0.00	0.00	0.34
Percentage of shallow habitat	−0.17	0.30	**0.56**
Canopy coverage (%)	0.29	−0.15	0.24
Coverage of submerged macrophytes in May	0.18	0.29	**0.85**
Coverage of submerged macrophytes in June	0.00	0.21	**0.89**
Coverage of submerged macrophytes in July	0.00	0.16	**0.90**
Coverage of floating leaved macrophytes in May	0.00	0.00	0.00
Coverage of floating macrophytes in June	0.00	0.00	0.00
Coverage of floating macrophytes in July	0.00	0.00	−0.12
Water temperature in May	−0.15	0.15	0.10
Water temperature in June	0.00	0.00	−0.21
Water temperature in July	0.00	−0.13	−0.18
Oxygen saturation in May	0.00	**−0.48**	−0.30
Oxygen saturation in June	0.00	−0.32	−0.17
Oxygen saturation in July	0.00	**−0.59**	0.00
pH	**0.42**	−0.13	0.00
Conductivity	**0.92**	0.24	0.00
Nitrate	−0.14	**0.35**	−0.28
Ammonia	0.11	**0.76**	0.18
Phosphorus	0.16	**0.83**	0.21
Calcium	**0.86**	−0.22	0.00
Sodium	**0.89**	0.25	0.20
Chloride	**0.88**	0.29	0.12
Iron	0.00	**0.71**	0.31
Manganese	0.37	**0.51**	0.36
Aluminium	−0.38	**0.52**	0.00
Potassium	0.25	**0.83**	0.32
Magnesium	**0.91**	0.13	0.00
Mann–Whitney *U*-test (*P*-value)	0.124	0.035	0.002

Important loadings are shown in bold type (see methods). *P*- values refer to Mann–Whitney *U*-tests comparing the scores of ponds with tadpoles present ($N = 19$) with those without tadpoles present ($N = 17$).

4.3. *Reproductive success and population size*

Long-term data suggest density dependent population regulation during the aquatic stage of some amphibians, and population fluctuations are therefore to be expected depending on reproductive success (Meyer et al., 1998). Our results also indicate that reproductive failure may limit population size of *P. fuscus* even in highly suitable habitats, including those with sandy soils and low traffic intensity. Thus, only about 50% of the sites investigated contained tadpoles in the summer of 2004. This is particularly worrying since the sites chosen were known to be among the best for the species as indicated by the number of calling males present in recent years (Berglund, 1998; Nyström et al., 2002). In fact, we found tadpoles in all ponds within a locality in only one of the nine studied areas (Smedstorp, Fig. 1, Table 1). It should also be noted that reproductive success at many of the sites with fewer than five calling males that were not included in our study is essentially unknown. Moreover, since *P. fuscus* has high site fidelity and typically lives in source-sink populations, with low exchange rates of adults between nearby ponds (about 1%, Hels, 2002), reproductive success is likely to be crucial for the long-term persistence of many populations. This is because the importance of successful reproduction for the persistence at a site of an amphibian population increases with degree of spatial isolation (Richter et al., 2003). Our correlative data indicate that reproductive success was more closely related to water quality and macrophyte coverage than to characteristics of the habitat near ponds, and is in agreement with the findings of other studies on amphibian reproduction in agricultural ponds (Knutson et al., 2004). For example, macrophytes play important and complex roles in aquatic environments by affecting predator-prey interactions, food availability and water chemistry (Jeppesen et al., 1998). For amphibians, including *P. fuscus*, macrophytes provide attachment sites for eggs (e.g. Strijbosch, 1979) and since submerged macrophytes increase habitat complexity, predation on tadpoles may decline (Axelsson et al., 1997). Thus, many amphibian species in farmland areas select ponds with a high coverage of submerged macrophytes as breeding sites (Ildos and Ancona, 1994; Laurila, 1998; Stumpel and van der Voet, 1998; Jansen and Healey, 2003). In accordance with our results, high coverage of submerged macrophytes has been linked to reproductive success in *P. fuscus* in Denmark (Hansen, 2002).

Recent studies have also shown that amphibian recruitment and species richness may be significantly reduced in farmland areas due to low hatching success associated with poor water quality; high levels of BOD (biological oxygen demand), nitrogenous compounds (e.g. ammonia) and phosphorus, and low coverage of submerged macrophytes (De Solla et al., 2002; Jansen and Healey, 2003; Knutson et al., 2004; Ortiz et al., 2004). Amphibians such as *P. fuscus* that live in agricultural landscapes, may be exposed to several environmental stressors associated with eutrophication, as indicated by this study, and possibly by the use of herbicides and pesticides. Even though these stressors may be toxic in many situations when studied in isolation (summarized in Rouse et al., 1999) they may contribute to even greater mortality when combined with predation threat or UV-B radiation (Relyea, 2003; Hatch and Blaustein, 2003; Broomhall, 2004; Teplitsky et al., 2005). Eutrophication may also alter food web structure in complex ways and affect amphibian survival, indirectly by inducing malformations associated with parasite infections (Johnson and Chase, 2004). Non-lethal effects may also affect survival in the terrestrial stage if feeding and subsequent size at metamorphosis is affected (Ortiz et al., 2004; Rohr et al., 2004).

Exposure to ammonium nitrate fertilizers may be toxic to adult *Rana temporaria*, but whether there also may be significant sublethal effects that indirectly influence mortality and reproduction of frogs is less well known (Oldham et al.,

Table 3 – Mean values (ranges) for the number of calling males and 32 environmental variables measured in and near ponds with (N = 19) and without (N = 17) tadpoles of *Pelobates fuscus* in 2004

Source	Tadpoles	No tadpoles
Maximum number of calling males	38 (1–120)	15 (0–70)
Agriculture (%)	24 (0–100)	48 (0–100)
Pasture (%)	73 (0–100)	49 (0–100)
Forest (%)	3 (0–60)	3 (0–40)
Maximum pond depth (m)	1.2 (0.5–2.0)	1.3 (0.5–3.0)
Pond area (m^2)	1020 (180–7000)	920 (160–4000)
Percentage of shallow habitat	69 (20–100)	65 (20–100)
Canopy coverage (%)	14 (0–30)	11 (0–60)
Coverage of submerged macrophytes in May (%)	30 (1–75)	20 (0–80)
Coverage of submerged macrophytes in June (%)	25 (5–80)	10 (0–70)
Coverage of submerged macrophytes in July (%)	25 (5–75)	10 (0–70)
Coverage of floating leaved macrophytes in May (%)	15 (0–75)	10 (0–65)
Coverage of floating macrophytes in June (%)	20 (0–85)	20 (0–90)
Coverage of floating macrophytes in July (%)	25 (0–80)	20 (0–90)
Water temperature in May (°C)	18.5 (16.1–23,9)	18.7 (16.4–22.6)
Water temperature in June (°C)	18.3 (15.0–24.0)	18.8 (15.4–21.3)
Water temperature in July (°C)	18.3 (13.4–21.1)	18.5 (16.3–20.7)
Oxygen saturation in May (%)	121 (69–165)	121 (23–176)
Oxygen saturation in June (%)	111 (49–188)	109 (8–225)
Oxygen saturation in July (%)	109 (38–152)	108 (9–187)
pH	8.2 (6.2–9.0)	8.2 (6.3–9.0)
Conductivity (mS/m)	28 (7–69)	47 (7–148)
Nitrate–nitrogen (mg/1)	0.003 (<0.001–0.027)	0.206 (<0.001–2.1)
Ammonia–nitrogen (mg/1)	0.074 (0.015–0.19)	2.37 (0.027–38.9)
Phosphorus (mg/1)	0.261 (<0.001–3.221)	1.58 (<0.001–20.52)
Potassium (mg/1)	8.1 (1.5–31.0)	22.4 (2.0–226.9)
Sodium (mg/1)	13.3 (3.4–49.7)	21.2 (3.8–78.6)
Chloride (mg/1)	26.5 (2.4–139)	44.2 (5.4–200)
Calcium (mg/1)	33.5 (4.9–73.5)	48.3 (3.3–135.6)
Iron (mg/1)	0.173 (0.014–0.516)	0.438 (0.017–4.621)
Manganese (mg/1)	0.033 (0.01–0.078)	0.063 (0.01–0.386)
Aluminium (mg/1)	0.061 (<0.001–0.362)	0.084 (<0.001–0.371)
Magnesium (mg/1)	4.3 (1.0–11.7)	7.2 (0.9–34.5)

Figures given for terrestrial surroundings refer to percentages of a ponds circumference located within 10 m from agricultural fields, pasture and forest.

1997). Overall, the effects of agriculture and eutrophication on amphibian populations are likely to be complex, and the upper lethal concentrations of particular compounds found in laboratory experiments may overestimate the ability of species to tolerate exposure in more natural settings. The effects of stressors also are likely to be dependent on the species and population. For example, tadpoles of *H. arborea* were extremely sensitive to ammonium nitrate compared to five other amphibian species in Europe (Ortiz et al., 2004). Johansson et al. (2001) showed that tadpoles of *R. temporaria* from northern parts of Scandinavia were more affected by nitrate than populations from the south, probably as a result of local adaptation. Although water chemistry may not be a good predictor of amphibian species richness, it may help to distinguish between sites that are used or not used by some species (Hecnar and M'closkey, 1996). For example, it is likely that *P. fuscus* actively avoid ponds containing predatory fish (Berglund, 1998; Nyström et al., 2002), but it does not seem to avoid breeding in eutrophic ponds (Hansen, 2002). This may be because the negative effects of eutrophication are not necessarily evident at the time of spawning (e.g. later development of low oxygen levels and low temperature associated with macroalgal coverage of the water surface), or because frogs cannot detect high concentrations of nutrients such as ammonia and nitrate. Nevertheless, Strijbosch (1979) who studied *P. fuscus* in the Netherlands, found that adults select the most eutrophic and well oxygenated breeding habitats (see also Nyström et al., 2002), and suggested that it was important for species whose larvae grow to a large size at metamorphosis to breed in productive waters. However, in that study some ponds with deposited eggs had ammonia concentrations above 2 mg/l, and all eggs-strings observed were infected by the fungus *Saprolegnia* every year for four years. In another study on *P. fuscus* in eutrophic ponds in Denmark, reproduction failed in about 50% of the ponds (Hansen, 2002) And a discriminant analysis indicated that total- nitrogen concentration and phytoplankton biomass were important factors separating ponds with reproduction from ponds without reproduction. Hansen (2002) also showed that survival of tadpoles in cages was significantly lower in hypereutrophic ponds than in eutrophic ponds. In our Scania study ammonia–nitrogen concentration was always below 0.2 mg/l and nitrate–nitrogen was below 0.03 mg/l in ponds with successful reproduction. On the other hand, the extreme values in ponds without frog reproduction were 38.9 and 2.1 mg/l, respectively. It is notable that the site with the highest

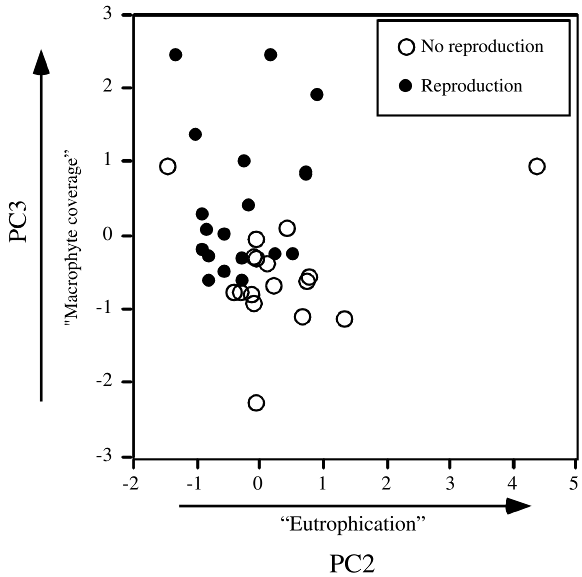

Fig. 4 – Component scores for the second and third principal components for sites with (N = 19) and without (N = 17) tadpoles of *Pelobates fuscus* in 2004. Originally 32 abiotic and biotic variables were included in the PCA. Variables with high loadings on axis 2 are associated with water quality ("eutrophication") and on axes three with the proportion of submerged macrophytes in shallow areas (Table 3).

concentration of ammonia–nitrogen was a site in a nature reserve with high densities of livestock (Tryde, Fig. 1), suggesting that high densities of livestock may have negative effects on pond water quality and vegetation, and consequently, reproduction in amphibians (Jansen and Healey, 2003; Knutson et al., 2004).

In our study, the discriminant function was better at classifying ponds without tadpoles (88%) than with tadpoles (68%) and indicates that variables such as coverage of vegetation and chemical indicators of eutrophication are more suitable for predicting conditions unsuitable for frog reproduction. On the other hand, our data indicate that other factors may need to be considered in order to correctly predict tadpole presence in *P. fuscus*. It is noteworthy that the two ponds without tadpoles that were misclassified were both located in the costal area of Vikhög (Table 2). Both these ponds (61–12 and 61–24, Table 2) had comparatively high concentrations of chloride (91 and 200 mg/l, respectively) and sodium (40 and 79 mg/l, respectively). However, the pond with the highest values for chloride and sodium but with tadpoles (61–11, Table 1) had concentrations of 50 and 139 mg/l, respectively. These results indicate that reproduction in *P. fuscus* may be negatively influenced by salinity in some ponds. Salinity tolerance of tadpoles is not well known but Lardner (2000) raised *R. arvalis* tadpoles originating from coastal areas on the east coast of Sweden at 2‰ NaCl and these tadpoles suffered higher mortality over 20 days than tadpoles raised in freshwater at 0‰.

4.4. *Implications for conservation*

Since ours was a correlative study it is impossible to determine the mechanisms behind our results. However, it is most likely that multiple abiotic and biotic stressors acting in concert contribute to the local and global decline of amphibians. The results of our study indicate that habitat destruction, high road traffic intensity and soil-type may affect the regional and local distribution of species such as *P. fuscus*, which are present in urbanized and agricultural landscapes. Furthermore, at sites where the species is still present population declines have sometimes been observed in areas with low traffic intensity and sandy soils. Our data suggest this could be because of low reproductive success, possibly because of tadpole mortality associated with agricultural activities. Since

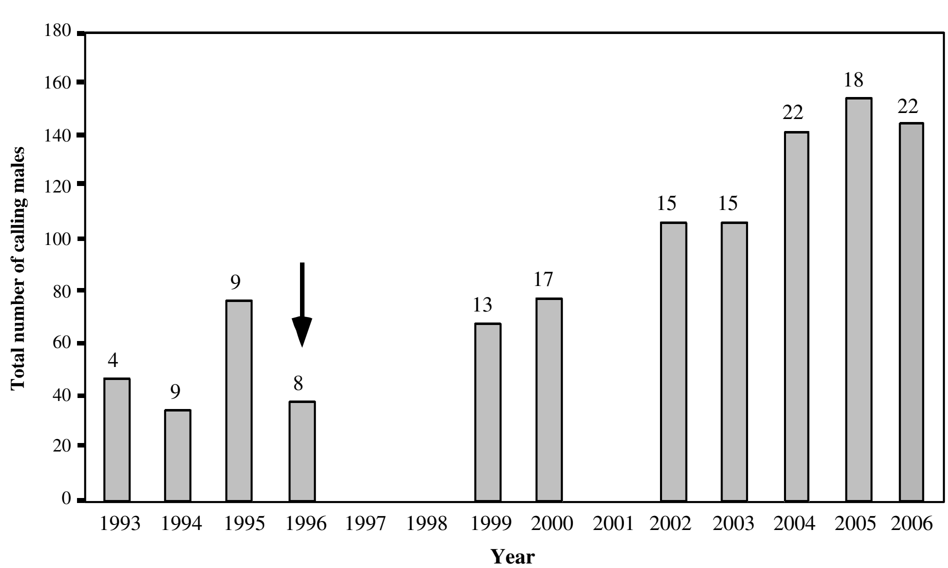

Fig. 5 – Total number of *Pelobates fuscus* calling males recorded in the Frihult area (Fig. 1) between 1993 and 2006. In 1996 pond restoration projects started (indicated by arrow). The total number of ponds with calling males present are indicated above the bar for each year. Note that data on calling males from the years 1997, 1998 and 2001 are missing.

Table 4 – Data on year of restoration of 19 ponds in Frihult (Scania) and the maximum number of calling males of *P. fuscus* recorded in different years

Pond	Restoration year	Category	1993	1994	1995	1996	1999	2000	2002	2003	2004	2005	2006	Other species in 2004
3	2001	R + D	0	1	0	0	0	0	1*	1	0	1	2	Tc, Tv
5	2001	R	0	0	0	0	0	0	0	2	3	1	1	Pf, Tc, Tv, Ha
9	2001	D	0	0	0	0	0	0	3	3	1	3	3	Pf, Ha, Ra
10	1996 (2005)	N (D + R)	–	–	–	–	5	6	5	5	4	0	1	Pf, Ha, Ra, Tc, Tv
11	2002	R + D	0	0	0	0	0	3	0	0	1	2	3	Pf, Ha, Rt, Ra, Tc, Tv
12	2002	R + D	0	0	0	0	0	4	13*	9	7*	10*	10	Pf, Ha, Bb, Rt, Ra, Tc, Tv
15	1999	D	0	0	0	0	0	0	1	0	1	1	2	Pf, Ha, Tc, Tv
17	1999	D	0	0	0	0	0	0	0	0	0	0	0	Tc, Tv
18	2001	D	0	0	0	0	0	0	0	0	4	0	4	Pf, Ha, Tc, Tv
22	1999	R + D	0	0	0	0	1	2	3	0	0	0	0	Ha, Rt, Ra, Tc, Tv
23	1999	D	0	1	1	0	0	0	0	0	6*	6	0	Pf, Ha, Rt, Ra, Tc, Tv
24	1999	R + D	0	4	3	2	0	2	2	0	10*	2	5*	Pf, Ha, Ra, Tc, Tv
25	1999	D	5	0	4	1	1	1	0	0	10	0	0	Pf, Ha, Bb, Rt, Ra, Tc, Tv
28	1996	N	–	–	–	–	0	0	0	2	2	0	0	Pf, Ha, Bb, Rt, Ra, Tc, Tv
30	2002	R + D	0	7	4	3	2	6	15	13	20*	30*	20*	Pf, Ha, Rt, Ra, Tc, Tv
31	1996	D	1	2	2	0	1	2	0	3	4*	1	4	Pf, Ha, Rt, Ra, Tc, Tv
32	1999	D	0	0	0	0	0	0	0	0	0	0	0	Ha, Ra, Tc, Tv
41	2001	N	–	–	–	–	–	–	0	0	0	0	0	Tv
42	2001	N	–	–	–	–	–	–	1	4	4	21	15	Pf, Ha, Bb, Tc, Tv

Category of restoration (N: new pond, R: removal of shading trees, D: deepened) are also given. Pond numbers in italics denote ponds with calling males prior to 1993 according to Berglund (1998). An asterisk denotes observations of *P. fuscus* tadpoles in June–July. All ponds were checked carefully for tadpoles and other amphibians only in 2004 (see methods). Pf: *Pelobates fuscus*, Ha: *Hyla arborea*, Bb: *Bufo bufo*, Rt: *Rana temporaria*, Ra: *Rana arvalis*, Tc: *Triturus cristatus*, Tv: *Triturus vulgaris*.

many of the ponds inhabited by *P. fuscus* are comparatively small (Table 4) they may be particularly sensitive to nutrient additions from agricultural runoff and defecating livestock. Direct livestock access to ponds has been shown to have negative effects on reproduction in many amphibians by affecting water quality and coverage of submerged macrophytes (Knutson et al., 2004). For ponds located in the agricultural landscape buffer zones need to be created to prevent access by livestock and to reduce the introduction of nutrients from the land.

To increase the probability that *P. fuscus* will increase in abundance at the local scale it is important that sites with successful reproduction be identified and given high priority in conservation planning. Furthermore, the mechanisms behind reproductive failure must be identified and the threat eliminated, especially for isolated populations (e.g. Törringe, Fig. 1). This is crucial if such populations are to avoid extinction in the near future because connectivity and dispersal of juveniles contributes more to regional persistence than adult dispersal in amphibians (summarized in Cushman, 2006). We assessed reproduction in one year only, and for long-lived species reproductive failure in one year may not be critical for population persistence (Richter et al., 2003). Studies over a longer period of time are therefore needed to assess the threat of reproductive failure for the persistence of *P. fuscus* populations. The restoration of ponds is likely to be one way to improve long-term survival in *P. fuscus* as indicated by our monitoring of the population in Frihult, and in Denmark where survival over five years of populations in restored ponds (82%), was much greater than in unmanaged ponds (32%) (Fog, 1997). We found that *P. fuscus* bred in new ponds, but new generations did not develop, possibly because submerged macrophytes had not yet colonized the ponds. Thus, new ponds may function as sinks in early stages. To further increase the probabilities of survival of *P. fuscus* and to prevent local declines, restoration of ponds should be directed towards areas with low traffic intensity, sandy soils and where the influence of agriculture and livestock access is low. Moreover, new ponds should be located near ponds with successful reproduction so frogs can reach them, easily. However, genetic factors should not be neglected since in natural amphibian populations most species are not driven to extinction before genetic factors impact them (recently reviewed in Frankham, 2005). Long-distance dispersal is often crucial to population spread and to maintenance of genetic connectivity and promotes long-term species survival (Trakhtenbrot et al., 2005). Since several of the remaining populations of *P. fuscus* in Sweden are comparatively small and isolated (e.g. Törringe and Kåsberga, Fig. 1), conservation strategies must consider the possible influence of inbreeding and loss of genetic variation in determining the long-term persistence of the species.

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