
GEOGRAPHICALLY VARYING HABITAT CHARACTERISTICS OF A WIDE-RANGING AMPHIBIAN, THE COMMON SPADEFOOT TOAD (*PELOBATES FUSCUS*), IN NORTHERN EUROPE

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Abstract.—Anthropogenic habitat loss and degradation are often cited as the primary causes of the recent decline or extinction of many species. The restoration of degraded habitats is therefore vital. Successful habitat restoration, however, requires proper identification of critical habitat characteristics and recognition of the factors that threaten the species. In this study, we describe the geographic variation of habitat characteristics for a widely distributed species with a declining population trend in Europe, the Common Spadefoot Toad (*Pelobates fuscus*). We examined 407 water bodies and their surrounding habitats in the Netherlands, Denmark, and Estonia by measuring 23 habitat characteristics and evaluating their effects on the species using canonical discriminant, logistic regression, and Spearman correlation analysis. We demonstrate that while the habitat features related to the selection of a breeding site by the species (based on presence/absence of larvae) were generally similar among the countries, habitat characteristics related to the quality of the breeding site (represented by larval abundance) varied considerably. In the Netherlands larval abundance correlated negatively with the area of uncultivated land and positively with the presence of organic crop fields near the breeding site. In Estonia, larval abundance was negatively related to deciduous forests in the surroundings of the reproduction site, and in Denmark it was mainly influenced by aquatic habitat qualities. Such differences could derive from the geographic variation of the habitat requirements of the species, but they could also indicate geographic differences in threatening factors present in each country. In the Netherlands and in Denmark, intensive agriculture seems to be the most important threat to the species, whereas in Estonia the overgrowing of open habitats (e.g., meadows, extensively used fields) and small freshwater bodies have severely affected the species.

Key Words.—Denmark; Estonia; geographic variation; the Netherlands; threats; threatened species

INTRODUCTION

Today almost one-fifth of extant vertebrate species, ranging from 13% of birds to 41% of amphibians, are classified as threatened (Hoffmann et al. 2010). The population declines are known or suspected to be caused by various anthropogenic factors, among which habitat loss and degradation are the most apparent culprits (e.g., Brooks et al. 2002; Stuart et al. 2004; Hoffmann et al. 2010). Restoration of degraded habitats is therefore increasingly critical to the recovery of threatened species (e.g., Greipsson 2011). Successful habitat restoration, however, requires the identification of critical (limiting) habitat characteristics and the factors that threaten the species (Whittingham et al. 2007; Hoffmann et al. 2010).

The habitat requirements of wide-ranging species may exhibit substantial geographic variation (e.g., Collins 1983), where habitat components critical for a species in one part of its range may be less important or even preferably avoided in another (Parody and Parker 2002;

Oliver et al. 2009; Rannap et al. 2012b). Likewise, the geographic variation of habitat requirements of a species may indicate geographic differences in threatening factors as shown by Jimu (2011) and Averill-Murray et al. (2012). It follows that study of the habitat requirements of a wide-ranging species in one area may be of little relevance to populations elsewhere. This is especially important for species of conservation concern.

Despite the need for studies exploring habitat requirements of species across their ranges, such studies are rare (but see Parody and Parker 2002; Rannap et al. 2012a). In this paper we describe the geographic variation in habitat characteristics of a widely distributed species, the Common Spadefoot Toad (*Pelobates fuscus*), and use this information to identify likely threats to it. According to the IUCN criteria, this pond breeding amphibian is classified as Least Concern (IUCN. 2009. The IUCN Red List of threatened species. Available from <http://www.iucnredlist.org/> [Accessed 10 December 2014]). However, its populations overall are

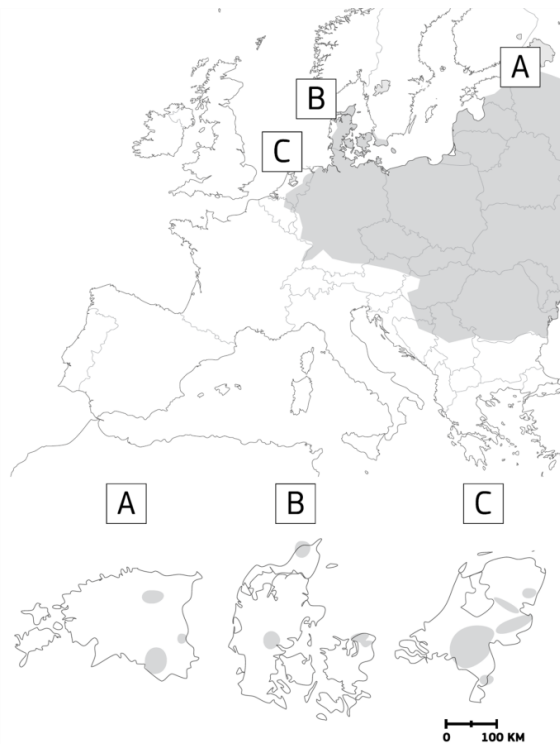


FIGURE 1. Distribution of the Common Spadefoot Toad (*Pelobates fuscus*) in Europe (shaded area above) and maps showing study areas (shaded areas below): A – in Estonia; B – in Denmark, C – in the Netherlands.

declining (Nöllert 1997) and the species is listed in the Annex IV of the EU Habitats Directive, requiring a strict protection regime (Council Directive 92/43/European Economic Community of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora. Available from http://ec.europa.eu/environment/nature/legislation/habitatsdirective/index_en.htm [Accessed 10 September 2015]). Such status demands conservation efforts and an explicit understanding of the habitat requirements to achieve a favorable conservation status across the range of the species. As amphibians have been identified as valuable models for ecological research when studying the impacts of habitat loss and degradation (Hopkins 2007), we believe that the results of our study may highlight issues critical for the conservation of other declining wide-ranging species.

MATERIALS AND METHODS

Fieldwork.—We conducted the study in three countries where the species has experienced a steady decline: the Netherlands, Denmark, and Estonia (Fog 1997; Van Delft et al. 2007; Briggs et al. 2008) between the latitudes 52°N and 59°N (Fig. 1). Over this latitudinal gradient, the growing season decreases from 293 d in the Netherlands (European Climate Assessment and Dataset. 2011. Available from <http://eca.knmi.nl>

[Accessed 5 January 2011]) to 225 d in Denmark (Christensen 2006) and 180 d in Estonia (Jaagus and Ahas 2000). To explore the essential habitat features for the Common Spadefoot Toad, we included both aquatic and terrestrial habitat characteristics in the study. As adult toads spend most of their terrestrial life in the vicinity of a breeding pond and rarely go further than 500 m from it (e.g., Nöllert 1990; Hels 2002), we used this distance to examine the landscape characteristics that influence the reproduction of the species. In the study we described 16 aquatic and seven terrestrial variables for water bodies and their surroundings (Appendix). We established the presence of fish as a combination of dip-netting (described below), visual observation, and information from local people.

We carried out the fieldwork in June 2010. We explored 407 water bodies and their surroundings: 170 water bodies in Estonia, 191 in Denmark, and 46 in the Netherlands. In Estonia and Denmark, we conducted the study in protected areas and their surroundings, covering the distribution area of the Common Spadefoot Toad. We preselected the water bodies, comprising small lakes, natural depressions, European Beaver (*Castor fiber*) ponds (only in Estonia), meanders, and man-made ponds created for cattle or garden watering, peat excavation, fish cultivation or for sauna use, from the base maps of Estonia (Estonian Land Board Geoportal. 2010. Available from <http://geoportaal.maaamet.ee/> [Accessed 15 August 2010]) and Denmark (Danish Natural Environment Portal. 2010. Available from <http://arealinformation.miljoeportal.dk/> [Accessed 26 August 2010]). Given the low abundance of the species in the Netherlands, we focused on sites where calling males of the Common Spadefoot Toad had been recorded by hydrophone at least once since 2000 (Wouter de Vries, unpubl. data). Aquatic and terrestrial habitat restoration (e.g., removal of mud, creation of organic crop and vegetable fields to provide high quality foraging and burrowing ground for the toad) had taken place in most of the Dutch study sites. In Denmark some amphibian-targeted pond management, influencing about 10% of the studied water bodies, had been carried out over the last 20 y. In Estonia such habitat management had not been implemented in the study sites.

To detect the presence/absence of the Common Spadefoot Toad larvae, we dip-netted the water bodies using a standard method (Skei et al. 2006). Each water body was visited once by a trained herpetologist and dip-netted on average for 25 min, covering all important microhabitats for amphibians. The dip-netting time varied from 10 to 32 min (SE = 6.4 min) and depended on the size of the water body. In smaller ponds we covered all microhabitats within 10 min, whereas in larger water bodies we dip-netted for longer (but not for more than 32 min). The same method also provided data

TABLE 1. The mean, minimum, and maximum values of continuous variables measured in the studied water bodies and their surroundings by country; F-statistic and P-value show the statistical significance of difference among countries according to analysis of variance (ANOVA).

Variable	The Netherlands (n = 46)		Denmark (n = 191)		Estonia (n = 170)		ANOVA	
	Mean	Min-Max	Mean	Min-Max	Mean	Min-Max	F	P
Area (m ²)	1,808.8	(30–15,000)	1,558.6	(10–11,700)	23,097	(28–856,800)	6.81	0.001
Shallow area (m ²)	556.8	(12–10,125)	410.5	(0–11,700)	1859.1	(0–88,900)	2.61	0.075
Max. depth (m)	1.37	(0.5–2.0)	1.62	(0.3–2.0)	1.67	(0.3–2.0)	5.67	0.004
Uncultivated area (m)	391.1	(1.4–3,352.5)	84.2	(0–800)	143.9	(0–625)	22.64	< 0.001
Average slope (°)	33.6	(2.5–90)	35.7	(3.8–90)	42.0	(2–90)	3.05	0.049
Shadow (%)	14.7	(0–75)	22.0	(0–100)	16.8	(0–100)	2.53	0.081
pH	-	-	7.22	(5.9–8.6)	7.44	(4.5–10.8)	4.35	0.038
Conductivity (mS/cm)	-	-	0.50	(0.16–1.04)	0.32	(0.09–0.99)	32.07	< 0.001
Water bodies <100 m	1	(0–3)	-	-	1	(0–4)	3.18	0.076
Water bodies 100–200 m	1	(0–4)	-	-	1	(0–5)	0.35	0.55
Water bodies 200–500 m	4	(0–12)	-	-	6	(0–19)	6.08	0.015
Forest edge (m)	56.9	(0–500)	73.7	(0–500)	26.8	(0–269)	12.24	< 0.001
Burrowing site (m)	12.9	(0–75)	47.9	(0–500)	36.1	(0–400)	3.10	0.049
Vegetation>1m (%)	11.0	(0–75)	21.56	(0–100)	8.73	(0–75)	14.17	< 0.001
Vegetation<1m (%)	19.04	(0–75)	12.33	(0–100)	17.04	(0–100)	3.10	0.046
Floating vegetation (%)	11.26	(0–75)	24.52	(0–100)	12.91	(0–100)	9.38	< 0.001
Submerged vegetation (%)	14.09	(0–75)	15.88	(0–100)	16.43	(0–100)	0.14	0.87
No. of amphibian species	3	(0–7)	2	(0–6)	2	(0–5)	27.55	< 0.001

on the relative abundance of the larvae (specifically, the total number of larvae counted) of the Common Spadefoot Toad, as well as on other amphibian species breeding in the same water body. Given the difficulties in distinguishing tadpoles of the Pool Frog (*Pelophylax lessonae*) and the Edible Frog (*P. kl. esculentus*), we refer to those species collectively as *P. lessonae/esculentus*.

Data analysis.—We conducted canonical discriminant analyses to construct the linear combinations of habitat characteristics (discriminant functions), distinguishing ponds with and without larvae of Common Spadefoot Toads. We entered the values of discriminant functions (canonical variables) as explanatory variables in a logistic regression with a binomial error distribution and a logit link to predict the occurrence of the larvae and estimate the prediction accuracy of the canonical variables. We also used Spearman's rank correlation coefficients to evaluate the association between the abundance of Common Spadefoot Toad larvae and each aquatic and terrestrial habitat variable. To estimate the effect of each aquatic and terrestrial habitat variable on the presence of Common Spadefoot Toad larvae we performed logistic regression analysis. Additionally, we conducted the analyses separately for each country because habitat characteristics differed among the three countries.

To determine if the assemblages of amphibian species for ponds with and without Common Spadefoot Toad larvae varied among the countries, we applied a principal component analysis on the abundance data of amphibian species. To test for differences in habitat characteristics between countries, we used ANOVA. To determine the importance of European Beaver ponds as breeding sites for the Common Spadefoot Toad in Estonia and to test for differences in the presence of fish between man-made and other pond types, we used χ^2 -test. To test for the differences in water conductivity between Estonia and Denmark we used the *t*-test. We considered correlations and differences significant at $P < 0.05$. We conducted all statistical analyses using the SAS 9.1 software (SAS Institute, Cary, North Carolina, USA).

RESULTS

We found breeding by the Common Spadefoot Toad in 11.2% of the Estonian, 11.5% of the Danish, and 28.3% of the Dutch water bodies. The characteristics of the water bodies differed among countries (Table 1). In Estonia larvae occurred significantly more often in beaver ponds than in other types of water bodies ($\chi^2 = 5.32$; df = 1, $P = 0.021$; Fig. 2). We found larvae mostly in small and shallow lakes (mean area 5,833 m²), with 60.0% (n = 3) of them having larvae in the Netherlands,

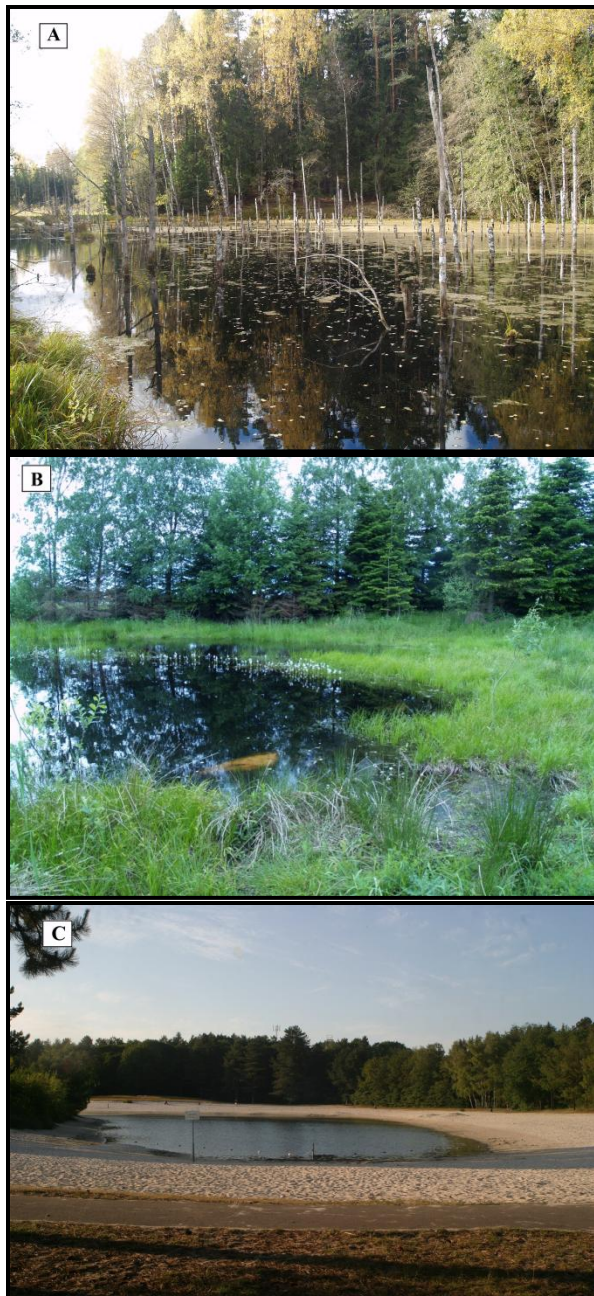


FIGURE 2. Natural water bodies used for breeding by the Common Spadefoot Toad (*Pelobates fuscus*): A – beaver pond in Estonia (Photographed by Riinu Rannap); B – natural depression in Denmark (Photographed by Riinu Rannap); C – shallow lake in the Netherlands (Photographed by Wouter de Vries).

and in natural depressions, with 15.5% ($n = 9$) of them having larvae in Denmark (Fig. 2). Although 60.0% ($n = 244$) of the available water bodies were man-made ponds in all three countries (Fig. 3), this type of water body was not preferred as a breeding site by the Common Spadefoot Toad in any of the countries ($\chi^2 = 41.71$, $df = 1$, $P \leq 0.001$ in Estonia; $\chi^2 = 80.71$, $df = 1$, $P \leq 0.001$ in

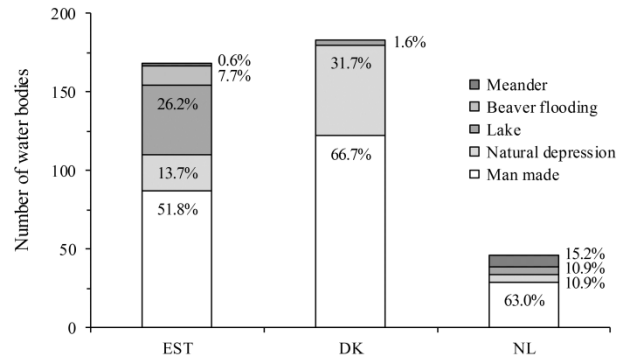


FIGURE 3. Frequency of water body type used by Common Spadefoot Toads (*Pelobates fuscus*) by country (EST- Estonia, DK – Denmark, NL – The Netherlands).

Denmark; $\chi^2 = 8.65$, $df = 1$, $P = 0.003$ in the Netherlands). There was no significant difference between man-made ponds and other types of water bodies regarding the presence of fish ($\chi^2 = 0.14$; $df = 1$, $P = 0.708$).

Aquatic habitat characteristics.—The presence/absence of larvae of Common Spadefoot Toads based on habitat characteristics did not differ among the countries (Table 2). In all three countries, base-rich sediment (clayish sediment favored; $\beta = 0.65$; $P = 0.036$ in overall logistic regression analysis) and large shallow littoral zones (water depth ≤ 30 cm; $\beta = 0.00022$, $P = 0.016$) had a positive effect on larval occurrence (Table 2). The correlations analysis supported the findings of discriminant analysis (Table 3). The absence of fish had a significant positive relationship with larval abundance of the Common Spadefoot Toad in Estonia and Denmark, but not in the Netherlands, where 15% of the breeding sites ($n = 2$) contained fish. Water conductivity, which correlated significantly with larval abundance in Denmark (Table 3), differed remarkably between the countries ($t = -5.04$; $df = 115$; $P < 0.001$), having lower mean value in Estonia than in Denmark (Table 1). Shade on the breeding site correlated positively with larval abundance only in the Netherlands ($r = 0.30$, $P = 0.042$). The area of shallow water ($F_{4,349} = 8.35$; $P < 0.001$), the conductivity ($F_{4,153} = 8.87$; $P < 0.001$), and the slope ($F_{4,371} = 16.37$; $P < 0.001$) differed significantly among the types of water bodies. The area of shallow water was the highest in lakes and beaver ponds than in man-made ponds. Man-made ponds also had the highest conductivity and the steepest slopes, whereas natural depressions had the lowest slopes.

Terrestrial habitat characteristics.—The presence of larvae was affected positively by open habitats and negatively by deciduous forests near water bodies in all three countries (Table 2). However, the type of open habitat in the surroundings of the breeding site had

TABLE 2. Results of canonical discriminant analyses (CDA) of the aquatic and terrestrial habitat characteristics measured.

Country Variables in CDA ^a	Netherlands		Denmark		Estonia	
	All	Selection	All	Selection	All	Selection
Prediction ability of canonical variables according to the logistic regression analysis ^b						
Sensitivity (%)	100.0	92.3	73.3	64.7	87.5	77.8
Specificity (%)	93.8	72.7	83.5	78.9	91.5	87.0
AUC	0.983	0.916	0.892	0.838	0.967	0.909
Description of canonical discriminant functions (CDF)						
Mean CDF (with larvae)	2.25	1.40	1.51	1.11	2.23	1.95
Mean CDF (without larvae)	-0.91	-0.55	-0.19	-0.14	-0.38	-0.29
Raw canonical coefficients ^c						
Type of water body						
Natural depression	-1.749	-	0.265	-	-1.851	-
Lake	2.475	1.892	-1.096	-	-1.479	0.098
Man-made	0.138	-	0	-	-1.382	-
Beaver pond	-	-	-	-	0	1.818
Meander	0	-	-	-	-	-
Aquatic characteristics						
Area (x10 ⁻⁴)	-4.358	-	-0.071	-	0.002	-
Shallow area (x10 ⁻⁴)	8.608	1.198	3.665	4.103	0.050	-
Maximum depth	0.318	-	0.477	-	0.400	-
Uncultivated area (x10 ⁻³)	-0.326	-3.960	-1.786	-	0.535	-
Average slope (x10 ⁻²)	-1.172	-	-0.730	-0.486	-0.770	-0.494
Shadow (x10 ⁻²)	0.552	-	-1.712	-	-0.910	-
Sediment						
Peat	-1.778	-	-0.717	-	-0.009	-
Mud	0.715	-	-0.349	0.122	0.038	-
Clay	2.536	1.944	0.556	1.001	0.643	0.335
Sand	0	-	0.521	-	0	-
Water						
Brown	3.505	-	1.490	-	-0.962	-
Clear	2.228	-	1.409	-	-1.405	-
Muddy	0	-1.041	1.274	-	-0.876	-
Algae-green	-	-	0	-	0	-
Number of water bodies						
<100 m	-0.238	-	-	-	0.274	-
100-200 m	0.394	-	-	-	0.229	0.169
200-500 m	0.206	-	-	-	0.062	-
Forest edge	0.0012	-	-	-	0.010	0.0107
Habitat within 50 m (presence)						
Coniferous forest	0.390	-	0.163	-	-0.214	-0.100
Deciduous forest	-0.178	-0.701	-0.200	-	-0.370	-0.573
Bogs/swamps	-	-	0.037	-	0.430	-
Crop field	2.294	1.354	0.394	-	0.016	-
Vegetable garden/field	1.034	1.394	-0.938	-1.191	-0.706	-
Gravel/sand pit	0.154	0.085	-	-	-	-
Meadow/fen	-1.112	-0.586	-1.098	-	0.611	0.391
Presence of fish	1.145	-	-0.688	-0.972	-1.478	-1.127

^a For different levels of categorical variables ("Type of water body," "Sediment," and "Water") numerical dummy variables were used. For each country two analyses were performed: first, all aquatic and terrestrial characteristics observed were involved ("All"); second, only characteristics showing higher prediction ability ($R^2 > 2.5\%$ in univariate ANOVA performed by SAS procedure CANDISC) were considered ("Selection"). Symbol "-" denotes the characteristics were not observed or did not vary in specific countries ("All"), or did not show the prediction ability over fixed threshold ("Selection").

^b Sensitivity and specificity indicate the proportion of water bodies with and without Common Spadefoot Toad larvae predicted correctly by canonical variable (the most optimal threshold corresponding to the maximum sum of sensitivity and specificity was used), respectively; AUC is the area under the ROC-curve;

^c Raw canonical coefficients are the multipliers of variables in discriminant function; in case of dummy variables and complete set of levels, they present the difference from the last level of present characteristic (these coefficients must be interpreted in conjunction with mean values of canonical discriminant functions in water bodies with and without larvae).

different effects on larval abundance in different countries (Table 3). In the Netherlands ecological crop fields, vegetable gardens/fields, and gravel/sand pits correlated positively and large areas of uncultivated land negatively with larval abundance (Table 3). In Estonia

the occurrence of a meadow/fen in the vicinity of breeding sites was positively correlated with larval abundance (Table 3). Additionally three terrestrial habitat features showed a positive correlation with larval

TABLE 3. Spearman rank correlation coefficients with *P*-values (in the brackets) between larval abundance of the Common Spadefoot Toad and habitat variables for the water bodies and surrounding habitats by country; statistically significant ($P < 0.05$) associations are presented in bold face.

Variable	The Netherlands (n = 46)	Denmark (n = 191)	Estonia (n = 170)
Type of water body			
Natural depression	-0.092 (0.539)	0.093 (0.209)	0.029 (0.703)
Lake	0.225 (0.132)	-0.046 (0.533)	-0.168 (0.029)
Man-made	0.019 (0.899)	-0.079 (0.284)	0.047 (0.544)
Beaver pond	-	-	0.159 (0.039)
Meander	-0.140 (0.351)	-	-0.027 (0.722)
Aquatic characteristics			
Area	0.167 (0.272)	0.117 (0.117)	0.053 (0.492)
Shallow area	0.072 (0.631)	0.164 (0.031)	0.198 (0.016)
Max depth	0.013 (0.931)	-0.092 (0.224)	-0.097 (0.220)
Uncultivated area	-0.334 (0.023)	-0.100 (0.199)	0.020 (0.810)
Average slope	0.113 (0.452)	-0.209 (0.005)	-0.130 (0.101)
Shadow	0.300 (0.042)	0.008 (0.914)	-0.096 (0.237)
Sediment			
Peat	-0.073 (0.627)	-0.024 (0.742)	-0.108 (0.171)
Mud	0.018 (0.902)	-0.082 (0.260)	0.012 (0.870)
Clay	0.169 (0.259)	0.107 (0.143)	0.174 (0.026)
Sand	-0.019 (0.900)	-0.008 (0.906)	-0.095 (0.228)
Water			
Brown	0.075 (0.617)	0.019 (0.787)	-0.026 (0.741)
Clear	0.057 (0.702)	0.017 (0.814)	0.070 (0.371)
Muddy	-0.158 (0.292)	-0.036 (0.619)	-0.089 (0.256)
Algae-green	-	0.000 (0.990)	0.139 (0.077)
pH	-	0.109 (0.341)	-0.134 (0.122)
Conductivity	-	-0.469 (0.006)	-0.120 (0.177)
Number of water bodies			
< 100 m	-0.188 (0.210)	-	0.042 (0.583)
100-200 m	0.072 (0.630)	-	0.165 (0.032)
200-500 m	0.067 (0.657)	-	0.040 (0.597)
Distance to the nearest			
forest	0.025 (0.866)	0.022 (0.833)	0.205 (0.007)
potential burrowing place	-0.060 (0.690)	0.024 (0.860)	0.360 (0.026)
Habitat within 50 m (presence)			
Coniferous forest	0.059 (0.693)	0.034 (0.647)	-0.124 (0.116)
Deciduous forest	-0.168 (0.261)	-0.136 (0.071)	-0.300 (<0.001)
Bogs/swamps	-	0.011 (0.875)	-0.074 (0.353)
Crop field	0.410 (0.004)	0.041 (0.583)	0.163 (0.040)
Vegetable garden/field	0.358 (0.014)	-0.146 (0.052)	-0.067 (0.395)
Gravel/sand pit	0.261 (0.079)	-	0.333 (0.666)
Meadow/fen	-0.190 (0.205)	-0.125 (0.098)	0.182 (0.021)
Vegetation in the water body			
Vegetation >1 m	-0.107 (0.479)	-0.091 (0.209)	0.083 (0.298)
Vegetation <1 m	-0.067 (0.655)	-0.037 (0.605)	0.048 (0.549)
Floating vegetation	-0.092 (0.541)	0.070 (0.335)	0.068 (0.397)
Submerged vegetation	0.176 (0.241)	0.094 (0.194)	0.083 (0.305)
Presence of fish	-0.060 (0.692)	-0.248 (0.001)	-0.356 (< 0.001)
Number of amphibian species	0.462 (0.001)	0.401 (< 0.001)	0.296 (< 0.001)

abundance in Estonia: (1) presence of crop fields near breeding site; (2) availability of burrowing sites (see Appendix for definition) within 50 m of a water body, and (3) number of water bodies near a breeding site (Table 3).

The canonical discriminant analyses of all aquatic and terrestrial habitat features correctly classified 87.5%, 73.3%, and 100% of the breeding sites of Common Spadefoot Toads from the Dutch, Danish, and Estonian water bodies (Table 2). The same analyses with selected

aquatic and terrestrial habitat features (according to their effect size) did not change the order of classification among the categories. The area under the ROC curve (AUC) indicated excellent prediction accuracy (AUC > 0.9) in the case of Dutch and Estonian water bodies and good prediction accuracy (AUC > 0.8) in the case of Danish water bodies, irrespective of whether all or only selected aquatic and terrestrial habitat features were used.

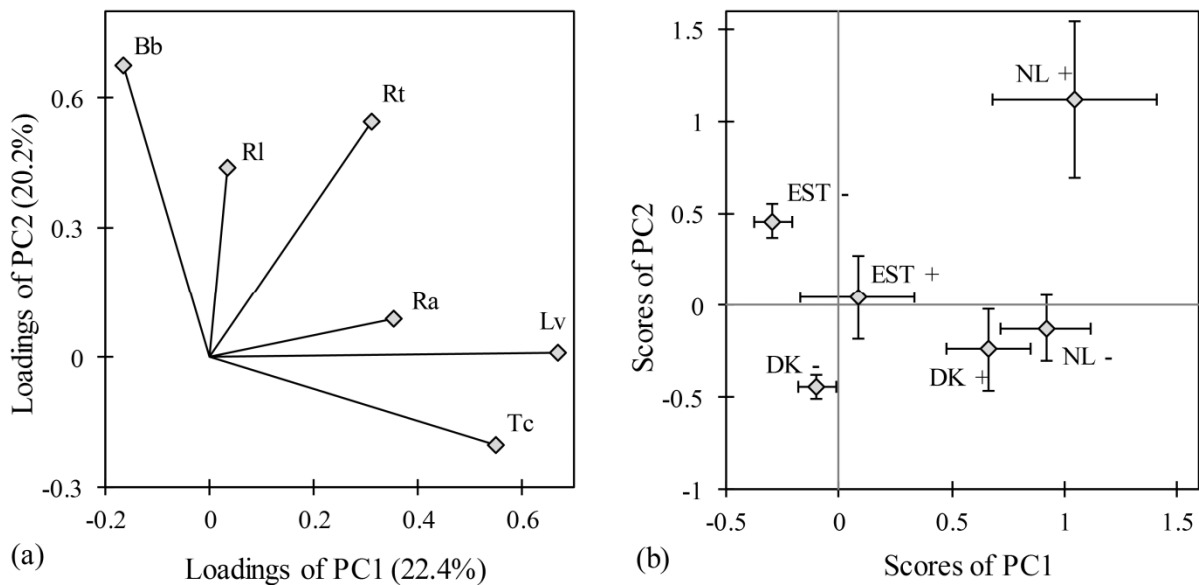


FIGURE 4. Principal component analysis plots of amphibian abundance. (a) Loadings of the first two principal components (PC); percentages in axis labels indicate the amount of the overall amphibian variation described by corresponding principal components (Bb - *Bufo bufo*, Ra - *Rana arvalis*, Rl - *Pelophylax lessonae/esculentus*, Rt - *R. temporaria*, Tc - *Triturus cristatus*, Lv - *Lissotriton vulgaris*). (b) Average PC scores (with standard errors) of Estonian (EST), Danish (DK), and Dutch (NL) water bodies with and without Common Spadefoot Toad (*Pelobates fuscus*) larvae (denoted as + and - in figure).

Amphibian diversity in the breeding sites.—In principal component analyses of abundance data of amphibian species, the first two components accounted for 42.6% of the total variance. The first component mainly represented the abundance of the Moor Frog (*Rana arvalis*), the Northern Crested Newt (*Triturus cristatus*), and the Smooth Newt (*Lissotriton vulgaris*), whereas the second component represented the abundance of the Common Toad (*Bufo bufo*), *Pelophylax lessonae/esculentus*, and the Common Frog (*R. temporaria*; Fig. 4a). From the score plot (Fig. 4b), it follows that in the Netherlands the water bodies with and without larvae of Common Spadefoot Toads were mainly separated in the vertical direction, indicating that larvae were generally present alongside larvae of *B. bufo*, *P. lessonae/esculentus*, and *R. temporaria*, whereas in Estonia and Denmark the water bodies with and without larvae of Common Spadefoot Toads were separated more in the horizontal direction, indicating that the species was associated with *R. arvalis*, *T. cristatus*, and *L. vulgaris*.

DISCUSSION

This study demonstrated that although the habitat characteristics related to breeding habitat selection of Common Spadefoot Toads (based on presence/absence data) were generally similar among the three countries, the habitat features related to the quality of breeding

sites (represented by larval abundance) varied among the countries. Such differences could derive from the geographic variation of habitat requirements of the species (e.g., Collins 1983), but they could also indicate geographic differences in threatening factors present in each country.

Aquatic habitat features.—Our study revealed that the habitat characteristics, which were important for the selection of breeding site of Common Spadefoot Toads, were largely similar in all studied countries in spite of the climatic differences. Although man-made ponds formed the majority of the examined water bodies in all three countries, the larvae were mainly found in natural waters with clayish sediment and large shallow littoral zones, indicating that pond quality may be more important for reproduction than pond availability (Denoël and Ficetola 2008). Man-made ponds had generally steeper slopes and a smaller area of shallow water than natural water bodies. However, when ponds are specifically constructed for amphibians in accordance with their habitat demands, they can successfully function as reproduction sites and can act as substitutes for natural water bodies, which are lacking in human dominated landscapes (Rannap et al. 2009a).

Clayish sediment assures clear transparent water, which also indicates high oxygen and low nutrient levels (Brönmark and Hansson 2005), both of which are vital for the species (Strijbosch 1979; Nyström et al. 2002).

Shallow littoral zones offer rapidly warming water and a diverse macrophyte cover, which provide suitable egg laying sites for adults and foraging and refuge sites for larvae, resulting in faster development rates (Semlitsch 2002; Porej and Hetherington 2005). The occurrence of large, shallow littoral zones could also explain the observed co-occurrence of fish and larvae of Common Spadefoot Toads in two of the Dutch breeding sites. Although presence of fish is considered a major limiting factor for pond-breeding amphibians (e.g., Hartel et al. 2007), in breeding sites with extensive shallow littoral zones and/or dense vegetation, such a coexistence may succeed (Hartel et al. 2007).

When larval abundance was taken into account, differences in habitat characteristics emerged among the countries. Shade on the breeding site had a positive effect on larval abundance only in the Netherlands. Although preference for sun-exposed breeding sites is vital for amphibians, including the Common Spadefoot Toad (Nyström et al. 2002; Rannap et al. 2013), breeding sites with shading can still be optimal habitats at lower latitudes where the growing season is considerably longer (Oldham et al. 2000).

Water conductivity, which was significantly negatively related to the abundance of Spadefoot larvae in Denmark, is often related to water quality. High conductivity may, among other things, indicate fertilizer pollution (Olías et al. 2008), which poses a serious threat to pond breeding amphibians (e.g., Oldham et al. 1997; Davidson et al. 2002). The significance of this habitat feature in Denmark but not in Estonia may be due to the generally higher water quality in Estonian sites. In Estonia only 20% of land is used for agricultural practices and large wilderness areas are still present (Peterson and Aunap 1998; Statistics Estonia. 2014. Environment. Available from <http://www.stat.ee/statistics> [Accessed 15 January 2015]). Thus, the impact of intensive agriculture is not as severe in Estonia as in many other European countries (Krebs et al. 1999; Donald et al. 2001), including Denmark (Hansen et al. 2001; Fox 2004). Unfortunately, the intensification of agriculture, especially the growing use of fertilizers, herbicides, and pesticides, is an ongoing process in Estonia. The severely negative impact of rapid agricultural intensification in new European Union member states, such as the Czech Republic and Poland, has already been demonstrated on birds and butterflies (Donald et al. 2001; Konvicka et al. 2006).

Terrestrial habitat features.—Terrestrial habitat variables that were related to the abundance of the larvae of Common Spadefoot Toads varied to an even larger extent among the three countries than the aquatic habitat attributes. Deciduous forests in the surroundings of the water bodies were negatively related to larval abundance in Estonia. This type of forest is often composed of

large amounts of dense undergrowth; vegetation the toad is known to avoid (Eggert 2002). The particularly negative impact of deciduous forests in Estonia probably results from the overgrowing of open landscapes due to land abandonment, followed by natural succession (Peterson and Aunap 1998) and reforestation (Soo et al. 2009). This trend is much more significant in Estonia than in the other studied countries. Avoidance of densely vegetated habitats by the toad may also explain why large areas of uncultivated land around breeding sites were negatively associated with larval abundance in the Netherlands, where such areas were often densely vegetated and covered in brushwood.

Open land cover types near the breeding sites had a positive impact on both larval presence and abundance in all studied countries. However, the preference for distinct habitats varied considerably between the countries. Meadows and fens near breeding sites were related positively with larval abundance in Estonia. These extensively used semi-natural grasslands provide open sun-exposed habitats and have remained quite natural (fertilizer free) in Estonia, as opposed to the Netherlands and Denmark, where meadows are regularly treated with artificial fertilizers (Emanuelsson 2009). The severe negative impact of the intensive use of grasslands, including fertilization of meadows and pastures, has been demonstrated on birds (Chamberlain et al. 2000; Vickery et al. 2001) and butterflies (Van Swaay et al. 2006).

Crop fields adjacent to the breeding waters (organic crop fields in the Netherlands and extensively used fields in Estonia) showed a positive association with the larval abundance of the Common Spadefoot Toad in both Estonia and the Netherlands, but not in Denmark. In contrast to most amphibians, this species has an advantage in agricultural habitats (Tobias et al. 2001) due to its foraging and fossorial behavior (Eggert 2002). However, the positive impact of crop fields in the two countries may have different causes. In Estonia where more than 50% of the total land surface is covered with forests and overgrowing/reforestation has a negative impact on the species, a general lack of open sun-exposed habitats increases the value of crop fields to the toads. In the Netherlands where agricultural land covers more than 60% of the total surface area and most of it is managed intensively (Oenema et al. 2005), organic crop fields and vegetable gardens established in the vicinity of the breeding sites of Common Spadefoot Toads provide high quality foraging grounds and burrowing sites for the toad.

Regarding the number of water bodies near the breeding sites, a higher number was positively related to the larval abundance of the larvae of Common Spadefoot Toads in Estonia. A clustered configuration of water bodies increases the probability of successful breeding and secures ecological connectedness and long-term

survival of metapopulations (Semlitsch 2002; Petranks et al. 2007). Generally, low numbers of high quality breeding sites available in the landscape might cause the importance of this habitat feature in Estonia alone. As demonstrated previously, in Estonia 78% of potential breeding waters are unsuitable for amphibian reproduction due to overgrowth, introduction of fish, or silting up (Rannap et al. 2009a).

Amphibian diversity in the breeding site.—Amphibian assemblages present in breeding sites along with the larvae of Common Spadefoot Toads differed remarkably from country to country. In the Netherlands the principal components analysis showed that Spadefoot larvae tended to occur in the same water bodies with *Bufo bufo*, *Pelophylax lessonae/esculentus*, and *Rana temporaria*, whereas in Estonia and Denmark the larvae were generally found alongside *R. arvalis*, *Triturus cristatus*, and *Lissotriton vulgaris*. Such differences in amphibian assemblages may reflect dissimilarities in breeding habitat quality between the countries: *B. bufo*, *P. lessonae/esculentus*, and *R. temporaria* are known to be species that to a certain extent tolerate intensively used agricultural landscapes (Loman and Lardner 2006), whereas *R. arvalis*, *T. cristatus*, and *L. vulgaris* tend to avoid such areas (e.g., Loman and Lardner 2006; Skei et al. 2006). The latter species also require breeding sites with clear transparent water and relatively low electrical conductivity (Skei et al. 2006), whereas the former can reproduce in freshwater bodies with variable habitat conditions (Ildos and Ancona 1994; Hartel et al. 2008).

Conservation implications.—Our study demonstrated that the habitat requirements of a wide-ranging species, the Common Spadefoot Toad, do vary among the studied countries. We therefore suggest that in the case of widely distributed species, information gained from different range states should be taken into account when restoring degraded habitats and constructing new ones. Moreover, the geographic variation of habitat requirements could also reflect differences in the factors that threaten the species in each country. Currently, intensive agriculture seems to be a severe threat to the Common Spadefoot Toad in the Netherlands and Denmark through the deterioration of the toads' aquatic and terrestrial habitats. We suggest that restoration and construction of aquatic habitats should therefore focus on high water quality (e.g., low conductivity and high oxygen levels) in those countries. Additionally, creating organic crop and vegetable fields in the vicinity of the breeding sites should also be considered there. In Estonia, however, intensive agriculture is not yet an acute threat to the species, enabling conservationists to take mitigation measures in advance. These could include designating protected zones around breeding

sites and restricting the use of agricultural chemicals, as well as supporting organic farming in the vicinity of the reproduction sites. In contrast to the Netherlands and Denmark, the overgrowing of open landscapes and water bodies has a negative effect on pond-breeding amphibians in Estonia. Open habitats (e.g., meadows, extensively used fields) should therefore be favored and deciduous forests avoided near the aquatic habitats. Studies conducted in several range states are particularly valuable because they allow for foreseeing the impact of possible threats yet unnoticed or absent in a particular country.

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Herpetological Conservation and Biology



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APPENDIX. Aquatic and terrestrial variables measured in the studied water bodies and their surroundings.

Variable	Variable Name	Method of Detection
<i>Aquatic variables</i>		
Type of studied water body: natural depression, lake, man-made pond, beaver pond, meander	Type of water body ^a	Detected in the field
Total area of water body (m ²)	Area	Measured from the base map ^b of the country or in the field (in case of small water bodies)
Mean area of shallow water zone (depth ≤30 cm), measured from four cardinal edges (m ²)	Shallow	Measured in the field
Maximal depth of water, measured by tape measure (m)	Max. depth	Measured in the field
Mean inclination of slopes of water body, measured from four cardinal edges (°)	Slope	Measured in the field
Predominant sediment type of studied water body. Four types were preselected: clay, sand, mud (distinguishable layer of organic matter), peat	Sediment ^a	Estimated in the field
Water transparency and color. Four types were preselected: clear (transparent water without color), brown (transparent water with brownish color), muddy (roily, turbid water), algae-green (roily water full of green algae)	Water ^a	Estimated in the field
Water pH	pH	Measured in the field by using pH-meter (PH-212; Lutron Electronic Enterprise CO., LTD. Taipei, Taiwan)
Water conductivity (µS/cm)	Conductivity	Measured in the field by using conductivity-meter (CD-4302; Lutron Electronic Enterprise CO., LTD. Taipei, Taiwan)
Proportion of water body under shadow of trees and/or bushes (%)	Shadow	Estimated in the field
Proportion of water body covered by >1 m high vegetation (%)	Vegetation >1m	Estimated in the field
Proportion of water body covered by < 1 m high vegetation (%)	Vegetation <1m	Estimated in the field
Proportion of water body covered by floating vegetation (%)	Floating vegetation	Estimated in the field
Proportion of water body covered by submerged vegetation (%)	Submerged vegetation	Estimated in the field
Presence of fish in water body	Fish	Determined in the field, combination of methods were used (see text)
Amphibian species presence	No of amphibian species	Determined by dip-netting of larvae (see text)
<i>Terrestrial variables</i>		
Mean width of uncultivated land around the water body, measured in four cardinal directions (m)	Uncultivated area	Measured from the base map of the country
Number of water bodies within 100 m of the studied water body	Water bodies <100 m	Determined from the base map of the country
Number of water bodies within 100–200 m of the studied water body	Water bodies 100–200 m	Determined from the base map of the country
Number of water bodies within 200–500 m of the studied water body	Water bodies 200–500 m	Determined from the base map of the country
Distance from the water body to the nearest forest edge (m)	Forest edge	Measured from the base map of the country
Distance from the water body to the nearest potential burrowing site – an area with sandy or loose soil (e.g., open sandy areas, pits, sandy road sites, gardens) (m)	Burrowing site	Measured from the base map of the country
Habitat within 50 meters, seven types were preselected: coniferous forest, deciduous forest, bogs/swamps, crop field, vegetable garden/field, gravel/sand pit, meadow/fen (presence) ^c	Habitat within 50 m	Determined in the field in the form: yes/no (1/0)

^a Categorical variables; for correlation and multivariate statistical analyses we formed dummy variables (1/0-variables) for each type of water body, sediment, and water (five dummy variables for types of water bodies and four dummy variables for both types of sediment and water) (Rannap et al. 2009b);

^b Base map for Estonia and Denmark, see text; for the Netherlands (<http://www.esri.nl/arcgis-content-basiskaarten> [Accessed 10 September 2010]);

^c Seven habitat types forming seven dummy variables in total.