Habitat requirements and conservation needs of peripheral populations:

The case of the great crested newt (*Triturus cristatus*) in the Scottish Highlands

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Abstract

Edge populations are of conservation importance because of their roles as reservoirs of evolutionary potential and in understanding a given species' ecological needs. Mainly due to loss of aquatic breeding sites, the great crested newt *Triturus cristatus* is amongst the fastest declining amphibian species in Europe. Focusing on the north-westerly limit of the *T. cristatus* range, in the Scottish Highlands, we aimed to characterise habitat requirements and conservation needs of an isolated set of edge populations. We recorded 129 breeding-pond related environmental parameters, and used a variable-selection procedure followed by random forest analysis to build a predictive model for the species' present occurrence, as well as for population persistence incorporating data on population losses. The most important variables predicting *T. cristatus* occurrence and persistence were associated with pond quality, pond shore and surrounding terrestrial habitat (especially mixed *Pinus sylvestris* - *Betula* woodland), and differed from those identified in the species' core range. We propose that habitat management and pond creation should focus on the locally most favourable habitat characteristics to improve the conservation status and resilience of populations. This collaborative work, between conservation agencies and scientific researchers, is presented as an illustrative example of linking research, management and conservation.

Keywords:

Edge population; amphibian; resilience; pond management; pond creation; collaborative work

Introduction

Fragmented peripheral populations are often reservoirs of genetic diversity, and play crucial roles in species' persistence (Channell & Lomolino 2000; Peterman et al. 2013). It is generally recognised that species' geographic ranges are determined by the interplay between history, climate and habitat, as well as life-history and physiology (summarised in e.g. Gaston 2009). However, we still have only a poor understanding of the specific biotic and abiotic factors which influence the persistence of populations at the periphery of a geographic distribution (Sexton et al. 2009). From the view of biological conservation, range-edge populations are worthy of attention for a range of reasons. Such populations are often morphologically and genetically distinct, and therefore important for preserving the full evolutionary potential of a species (Eckert et al. 2008; Lesica & Allendorf 1995). However, edge populations are often rather small and therefore exposed to high extinction risks through stochastic events, and a reduced amount of neutral genetic variation can further reduce their ability to persist (Sagarin & Gaines 2002; Sexton et al. 2009). The combination of small population size and a peripheral location can also limit the potential of populations to adapt to changing local environmental conditions (Bridle & Vines 2007; Kawecki 2008). An important, but rather underreported, consideration for the conservation management of peripheral populations is that the ecological niche space occupied by a given species can vary across its range, with habitat requirements as quantified through species-environment relationships therefore depending on geographic location (Pearman et al. 2010).

Most Palaearctic amphibian species breed in small water bodies, such as ponds, using adjacent terrestrial areas as summer foraging habitat for hibernation, migration, and dispersal; the use of confined breeding foci makes them very amenable for studies at the level of populations (Jehle et al. 2005; Petranka et al. 2004; Semlitsch 2008). Whether an available pond is occupied largely depends on a species' ecological requirements, as well as the degree of connectivity to other ponds (Ficetola & De Bernardi 2004; Halley et al. 1996; Marsh & Trenham 2001; Van Buskirk 2005). In recent decades, significant attention has been devoted to the use of habitat parameters for predicting the suitability of ponds and their surroundings for specific species (e.g. Denoël & Lehmann 2006; Hartel et al. 2007; Joly et al. 2001; Knapp et al. 2003). Habitat requirements, however, vary considerably across a species' range (Arntzen & Themudo 2008; Gomez-Mestre & Tejedo 2003; Zanini et al. 2009), and such studies can therefore convey a view which is biased towards the core of a distribution, or to particular environments such as agricultural landscapes (Hartel et al. 2010b; Mazerolle et al. 2005). As a result, despite the importance of peripheral populations for conservation, predictive habitat models calibrated to landscapes typical of central populations might be of limited value elsewhere.

The great crested newt, *Triturus cristatus* (Laurenti, 1768), is protected under Annex II and Annex IVa of the European Habitats Directive. Although still widespread, *T. cristatus* is amongst the fastest declining amphibian species in Europe; its conservation status is assessed as favourable in only 2 out of 22 European countries (Luxembourg and Denmark), a fact which has been linked to habitat loss (Denoël 2012; for a summary see Jehle et al. 2011; Rannap et al. 2009). At the core of its range, *T. cristatus* generally occupies both natural and artificial ponds in pastures and deciduous or mixed woodland, with pond macrophyte cover and connectivity being the most important parameters for predicting occurrence (Denoël et al. 2013; Halley et al. 1996; Hartel et al. 2010a; Hartel & von Wehrden 2013). At the northern periphery of its range (e.g. Scandinavia), however, typical habitats for *T. cristatus* include acidic bog lakes surrounded by coniferous woodland (Dolmen 1980; Skei et al. 2006; Vuorio et al. 2015). As current conservation management practices for *T. cristatus* are heavily based on habitat suitability models (Oldham et al. 2000; Unglaub et al. 2015), this raises the need to consider specific ecological, biogeographical and social contexts for assessing habitat requirements across its range (see also e.g. Cayuela et al. 2016; Sjögren-Gulve 1994 for other amphibian species).

Triturus cristatus reaches its north-westerly limit in the Scottish Highlands, where a set of populations is separated from the remainder of the British range by over 80 km of unfavourable habitat. Due to this spatial isolation, it was previously assumed that these populations stem from introductions, and their native status was only recently demonstrated using genetic means (O'Brien & Hall 2012; O'Brien et al. 2015). The aim of the present study is to employ the case of *T. cristatus* in the Scottish Highlands as a model to describe the habitat requirements of, and the effect of human activities on, a European flagship wetland species at the edge of its distribution. In order to achieve this, we used a detailed dataset of 129 ecological variables to compare occupied with unoccupied ponds, also considering ponds with reported disappearance events. Our approach to establishing local habitat requirements differs, for example, from the existing Habitat Suitability Index for this species (Oldham et al. 2000) by incorporating a much larger (>10 fold) number of variables, which should enable the description of local ecological needs of *T. cristatus* in more detail. Besides serving as an example for elucidating different ecological niches across the range of a species of considerable conservation importance, the study has already been used to inform habitat creation and management measures instigated by the local government agencies.

Materials and Methods

Study area and field survey

Field work for this study built upon volunteer surveys starting in the late 1980s, as well as *ad hoc* records extending back to 1896 (NBN 2014). We considered 88 ponds in total, which encompassed all 33 known ponds in the Scottish Highlands with *T. cristatus* records since 1990 (excluding known introductions), seven ponds with populations found during the present study, and 48 control ponds without *T. cristatus* occurrence. Control ponds were located within the same group of 10x10 km squares as the known *T. cristatus* ponds, chosen using a random number generator to select grid references (4º35'–3º35'W, 57º38'–57º11'N; Fig. 1). The studied ponds represented glacial and man-made sites, the age of the latter ranging from prior to the earliest detailed maps (surveyed c. 1870) to ponds created within the last 10 years. Altitudes ranged from 10 m to 248 m a.s.l. (median 91.5 m).

We surveyed each pond to determine the presence of *T. cristatus* at least three times per year, using four techniques following the British National Amphibian and Reptile Recording scheme (NARRS) protocol: egg searching, dip netting, torching, and trapping (ARG-UK 2013; Griffiths & Langton 2003; Langton et al. 2001). Egg searching involved looking for folded leaves, containing eggs, among the submerged vegetation. Dip netting was carried out from the shore using a net with a 2 mm mesh, sweeping the whole perimeter of ponds smaller than 3000 m^2, and at least 300 m of shoreline, including all habitats present, for ponds larger than 3000 m^2. Torching (Cluson Clulite CB2, 1 million C/P) was conducted from shortly after dusk to shortly after midnight, walking around the entire pond perimeter. Trapping was carried out using up to 20 46x21x21 cm funnel traps for each pond (4 mm nylon mesh with 6 cm diameter openings at each end, see Madden & Jehle 2013). Funnel traps were installed amongst aquatic plants shortly before sunset and checked within 10 hours. Data from surveys were pooled to determine the presence or absence of *T. cristatus* at given ponds.

All surveying followed Scottish Natural Heritage guidance, to ensure welfare of newts and non-target species, and the disease and non-native species control measures advised for amphibian field workers (ARG-UK 2008).

Habitat descriptors and data analysis

To investigate which habitat features were most important to predict the presence of *T. cristatus*, we collected data from 129 variables, 88 derived through field work and 41 through desk study after the field sampling period. Topographical features were obtained from GIS using 1:25 000 maps from the British mapping agency Ordnance Survey. Water-associated variables were gathered in the field by handheld devices or estimated using semi-quantitative scales. Anthropogenic activities, the aquatic macroinvertebrate community, the vegetation communities, and other habitat characteristics of the ponds and their surroundings were assessed using percentages or semi-quantitative scales. Given the conservation management context of the study and the proximity of occupied and non-occupied control ponds, we did not include spatial autocorrelation variables to avoid unnecessarily complexity and collinearity in the models. For further details on habitat descriptors see Online Resource 1.

We assessed the habitat requirements of *T. cristatus* applying two successive statistical approaches: variable selection by individual binomial tests and variable exploration by principal component analysis (PCA), followed by establishing the relative importance of the selected predictor variables by non-parametric random forest analyses (see below). Each statistical procedure was performed separately to investigate both *T. cristatus* presence or absence (occurrence analyses), as well as population persistence (persistence analyses). We defined lack of persistence as failure to record *T.cristatus* since 2010 despite previous records, based on annual surveys consisting of at least three visits each.

Individual binomial tests were made on each of the 129 variables to determine their relevance to *T. cristatus* occurrence and persistence. Mann-Whitney U tests and Chi-square (χ^2) tests were used for numerical and categorical variables, respectively. Individual Generalized Additive Models (GAM) for every original variable were also produced with the same objective. Significant variables ($P < 0.05$) were grouped and examined for collinearity. We excluded numerical and categorical variables which had a Variance Inflation Factor (VIF) >3.0, and numerical variables which showed Pearson pairwise correlations >0.6 (Tables 2-7 in Online Resource 1 Zuur et al. 2009). The remaining significant predictor variables were represented by PCAs, where categorical variables were used directly as dummy (1/0) transformed variables.

We then investigated the relative importance of the selected predictor variables using random forest classification analyses (Breiman 2001; Cutler et al. 2007). Random forest analysis generates multiple classification (or regression) trees, using a predefined number of random variables for each split. At the end of the process, the importance of the variables is estimated based on the frequency at which each variable has been chosen as the best in all trees (or the average value for regression trees). Specifically, we produced non-parametric unbiased recursive random forests (Hothorn et al. 2006), where the selection of the best split is based on conditional inference tests, to avoid bias in favour of continuous variables and variables with many categories. The number of trees was specified as 500, and the number of random preselected variables in each split was the square root of the total number of available variables (e.g. Hapfelmeier & Ulm 2013). Individual Variable Importance Measures (VIM) were computed through an algorithm which uses the area under the curve (AUC) as a measure of accuracy, which is robust against class imbalance of the response variable (Janitza et al. 2013).

Given the high number of modelled variables in comparison to the number of ponds in our dataset, the statistical procedure we followed offered more accuracy and was easier to interpret than other methods such as generalized linear or additive models (GLM/GAM). We refrained from performing occupancy modelling, since the comprehensive surveys to confirm the presence of *T. cristatus* provided consistent results across the six breeding seasons.

All analyses were performed with *R* statistical software (R Development Core Team 2014) using the basic functions and the packages *MASS* to compute χ^2 tests (Venables & Ripley 2002), *mgcv* to perform and plot individual GAMs (Wood 2011), and *party* to produce random forests (Hothorn et al. 2015). Numerical variables were normalized before being introduced in random forest analyses when necessary.

Results

General characteristics of the ponds

The investigated ponds had a maximum water depth ranging from 0.1 m to 4.5 m (median 1.0 m), surface areas of 2 m^2 to 164 500 m^2 (median 1615 m^2), and perimeters ranging between 5 m and 3 127 m (median 221.5 m). Conductivity ranged between 8.8 and 441 µS/cm (median of 100.6 µS/cm), and pond water pH was between 3.42 and 9.52 (median 6.34). Dissolved oxygen concentrations ranged from 1.0 to 18.3 mg/L (median 9.4 mg/L), leading to oxygen saturations of 10-180% (median 87.5%). None of these characteristics showed statistical differences between ponds with and without *T. cristatus* (Fig. 4 in Online Resource 1).

Triturus cristatus presence

We confirmed the presence of *T. cristatus* in 24 of the 33 ponds with post-1990 records, and found seven new *T. cristatus* ponds, resulting in a total of 31 ponds where *T. cristatus* was present. All newly discovered populations were within one kilometre of at least one previously known, occupied pond. *Triturus cristatus* could not be detected from the remaining 57 study ponds, which comprised 48 unoccupied ponds (where *T. cristatus* has never been recorded) and nine ponds where *T. cristatus* was previously recorded but not found over the six years of our study.

Occurrence analyses

After accounting for collinearity, 12 predictor variables (eight numerical and four categorical) were selected as significantly related to *T. cristatus* occurrence (Table 1; Fig. 5 & 6 in Online Resource 1). Individual GAMs identified nine habitat quality variables that were positively related to a high probability of *T. cristatus* presence. These were: adjacent mixed *Pinus sylvestris* - *Betula* woodland (EUNIS category G4.4, European Environment Agency 2014), substrate of organic mud, macroinvertebrate richness, slightly sloping bank, aquatic macrophytes (except aquatic mosses and *Lemna* sp.), soils with humus-rich iron podzols, terrestrial habitat diversity, underlying geology of sand and gravel, and moss coverage within 1 m of the water's edge (Fig. 6 in Online Resource 1). Presence of fish and underlying geology of boulder clay decreased the probability of occurrence of *T. cristatus*. The probability of *T. cristatus* presence also decreased when ponds frequently dried up (more than twice in ten years), although occurrence was highest in ponds drying once every ten years (Fig. 6 in Online Resource 1).

PCA representation showed that the variables associated with habitat quality were strongly correlated with the first principal component, which represented 18.7 % of the explained variance and allowed a good discrimination between ponds with and without *T. cristatus* (Fig. 2a). Presence of fish was the best predictor variable of the second principal component (16.4 % of the explained variance), combining most of the ponds where *T. cristatus* was absent (Fig 2a). Although only 35.1% of variance is explained by the two principal components, the graphic accurately represents the relationship between pond characteristics and significant explanatory variables.

The random forest model had a misclassification error of 12.5%, an out-of-bag mean error of 28.4% and a 10-fold cross validation mean error of 26.2%. In general, the variables most associated with the principal components of the PCA showed the highest values of variable importance measures (VIM). In addition to fish presence, variables related to habitat quality were most important: adjacent mixed *Pinus sylvestris* - *Betula* woodland, substrate of organic mud, macroinvertebrate richness, years when the pond dries up and slightly sloping bank. However, fish presence was the second most important variable. Variable Importance Measures (VIM) are shown in Fig. 3a and in Table 8 of Online Resource 1.

Persistence analyses

Through the individual tests, and after accounting for collinearity, eight predictor variables (seven numerical and one categorical) were selected as significantly related to *T. cristatus* persistence (Table 1; Fig.7 & 8 in Online Resource 1), with individual GAMs highlighting five habitat quality variables positively related to *T. cristatus* persistence: grass coverage of the shore, macroinvertebrate richness, the second principal component for the set of human activities, adjacent mixed *Pinus sylvestris* - *Betula* woodland, and moss coverage of the shore. The second principal component of the stressor structure was positively correlated with the level of shooting (Spearman's rho = 0.352, $P = 0.026$), and negatively correlated with the high presence of roads surrounding the ponds (rho = -0.711, $P < 0.001$) as well as noise (rho = -0.580, $P < 0.001$), which was itself strongly correlated with the presence of surrounding roads (Spearman's rho = 0.37, $P = 0.009$; Table 9 in Online Resource 1). Presence of fish also lowered the probability of *T. cristatus* persistence, whereas there was no clear relationship with pond drying (Fig. 8 in Online Resource 1).

The PCA revealed that the first axis represented 30.8% of the explained variance, with a good discriminatory ability between ponds with permanent occupancy and ponds where *T. cristatus* had disappeared (Fig. 2b). The variables connected with shore habitat quality were most strongly correlated with the second principal component (18.9% of the explained variance), which also identified the ponds where *T. cristatus* has disappeared. Presence of fish was correlated with both axes (Fig. 2b).

The random forest model produced for *T. cristatus* persistence had a misclassification error, an out-of-bag mean error and a 10-fold cross validation mean error of 22.5% each. Grass coverage of the shore was the most important habitat feature, linked to macroinvertebrate richness, pond drying and tree coverage of the shore. Fish presence was the second most important variable, followed by the second principal component for the stressor structure (mainly correlated with the high presence of roads surrounding the pond). Variable Importance Measures (VIM) are shown in Fig. 3b; and in Table 10 of Online Resource 1.

Discussion

Management and conservation of *Triturus cristatus* peripheral populations

We have developed a detailed ecological model to illuminate the occurrence and persistence of a flagship species at the edge of its distribution. We showed that habitat characteristics favouring *T. cristatus* in the Scottish Highlands noticeably differ from its core range, while previously described negative predictors for occurrence, such as fish presence, exert similar adverse effects regardless of local habitat preferences (see also e.g. Cayuela et al. 2016; Sjögren-Gulve 1994 for other amphibian species). Our inferences are informing habitat creation and population management.

Our model, based on 129 habitat parameters, is amongst the most comprehensive datasets applied to predicting the occurrence or persistence of any amphibian species (compare e.g. Joly et al. 2001; Knapp et al. 2003). Such studies rely on accurate information on the presence or absence of a given species, which is a function of sampling effort and detection probability (e.g. MacKenzie et al. 2003; Schmidt 2005). Employing a nationally recognised protocol to record the presence of *T. cristatus* ensured comparability with other UK studies, but does not guarantee accurate detection across all ponds (see discussion in Griffiths et al. 2015). However, our presence/absence findings at individual ponds were consistent across the six-year period of the study, suggesting that our records of population loss represent true demic extinctions. Another potential confounding factor when describing habitat relationships is spatial autocorrelation (e.g. Ficetola et al. 2015). Our predictive models did not explicitly include, for example, information on the distance to the nearest occupied ponds, since our analysis was geared towards active habitat management for conservation. The confined range of our study area further suggests that spatial autocorrelation is likely to have had little effect on our inferences (e.g. Griffiths et al. 2010).Previous studies on *T. cristatus* have also shown that, at similar spatial scales to those studied herein, demographic properties of populations play a more important role in shaping patterns of gene flow than terrestrial habitat characteristics (un)favourable for migration (Jehle et al. 2005b). We nevertheless plan to incorporate inter-pond terrestrial habitat variables more explicitly in future studies.

The vulnerability of *T.* cristatus in the Scottish Highlands stems from the small number of occupied ponds and their isolation from the species' core range, and its recent recognition as a native species makes its conservation a priority for government agencies (O'Brien et al. 2015). Long-term effectiveness of conservation interventions, such as pond creation and habitat management, relies on a thorough understanding of the species' habitat requirements at this part of its range. The relationship between population loss and fish presence is a particular concern, due to local pressure from recreational angling. New pond creation is focusing on sites where landowners support amphibian conservation and understand the dangers of fish, and on locations with low risk of fish introduction (e.g. avoiding roads and established fishing lakes). Whilst fish eradication has been apparently successful at one of the Highland ponds (O'Brien unpublished data), this may not always be practical.

Pond creation is a relatively inexpensive form of habitat management (Baker et al. 2011), although it utilises otherwise productive farmland or forest. An understanding of favourable habitat characteristics informs better pond design and creation. In the Scottish Highlands, we recommend pond creation close to mixed *Pinus sylvestris* - *Betula* woodland, on humus-rich iron podzols with underlying sand and gravel and away from busy roads. Surrounding terrestrial habitat should be managed to favour grassy and mossy shores. Informed by the data presented here, Scottish Natural Heritage and Forestry Commission Scotland created or modified 25 ponds in 2014 and 2015, of which three were colonised by *T. cristatus* within 14 months (in preparation).

Predicting *Triturus cristatus* occurrence

Our empirical data confirmed that habitat preferences of edge populations in the Scottish Highlands differ from the core of the species' range. Elsewhere (excluding Scandinavia) *Triturus cristatus* is associated with deciduous woodland and arable land, along with artificial breeding sites such as ponds dug for livestock or associated with mineral extraction (Beebee & Griffiths 2000; Jehle et al. 2011; Latham et al. 1996; Swan & Oldham 1993). Our study showed a strong link with mixed *Pinus sylvestris – Betula* woodland. Use of pine forest in northern regions was also shown by Skei et al. (2006), but was somewhat unexpected in our study as sizeable areas of deciduous woodland are present. The locally thermophilic *T. cristatus* may benefit from the relatively high ground level incident solar radiation afforded by the open canopy of *Pinus sylvestris – Betula* woodland, compared to the denser canopy of the dominant local deciduous woodland types acidophilus *Quercus*-dominated woodland and meso- and eutrophic *Quercus, Fraxinus, Acer, Tilia, Ulmus* and related woodland (EUNIS codes G1.8 and G1.A, respectively, European Environment Agency 2014). If the Scottish Highland population represents an isolated group of colonists (O'Brien & Hall 2012), then it seems likely that they are adapted to the dominant British habitat at the time of colonisation (Edwards & Whittington 2003). Thus, their phenotypic traits may differ from the main population (as observed for other amphibians, e.g. Rollins et al. 2015).

In contrast to previous evidence (Klinge 2001), we found a negative association between *T. cristatus* and clay, and a corresponding positive association with humus-rich iron podzols, substrates which are common in the study area. Organic mud, an important breeding area for potential food species, was also positively associated with *T. cristatus* presence. The prominent role of substrate in our models contrasts with a study from north-eastern Europe (Rannap et al. 2009), The strong negative relationship with fish presence agrees with previous studies, although we could not confirm a negative association with waterfowl (Oldham et al. 2000).

The frequency of pond drying proved important in predicting *T. cristatus* occurrence , although the relationship appears complex; drying in 1/10 years was most favourable (confirming Griffiths 1997; Oldham et al. 2000). *Triturus cristatus* bred in ponds with pH between 4.9 and 9.3, demonstrating use of more acidic ponds than elsewhere in Britain (Denton 1991, found adults in ponds with pH 4.7, but did not observe breeding), and a wider range than found at other northern limits (Dolmen 1980; Skei et al. 2006). Other factors positively correlated with *T. cristatus* presence were macroinvertebrate richness, slightly sloping bank, aquatic macrophytes (except mosses and *Lemna* sp.), moss coverage of the shore, and terrestrial habitat diversity (largely confirming Green 1984; Gustafson et al. 2006). In contrast to studies from other areas pond shading had no influence on presence or absence (Filoda 1981; Oldham et al. 2000), and negative effects from agriculture and forestry (presumed anthropogenic stressors) were not observed. Agricultural runoff and grazing pressure may be less of an issue in our region, where land management is less intensive than elsewhere in Western Europe. Potential negative impacts of commercial forestry may have been mitigated, or even reversed, through collaborative work with forestry agencies to manage habitat for amphibians (e.g. Forestry Commission Scotland & Scottish Natural

Heritage 2009). Water abstraction and other artificial changes in water levels, which have strong negative effects on amphibians and other aquatic biodiversity elsewhere (Miró 2016), are uncommon in the study area, as is mineral extraction. Whilst shooting had been considered a possible stressor, it was positively correlated with presence (P<0.03). This may reflect both habitat management for quarry species that also favour *T. cristatus*, and deer-culling in woodland for wider conservation and commercial benefit. As hunting is economically important across the species' range, this finding may merit further investigation.

Predicting *Triturus cristatus* population losses

Conservation of a species found in a small number of sites depends on an understanding of the reasons for its disappearance from previously occupied sites. Fish presence, or introduction, has been linked to *T. cristatus* disappearance (for a review see Jehle et al. 2011), as has presence of roads for amphibians in general, through direct mortality and population isolation (for a review see Beebee 2013). While noise was associated with disappearance linked to road presence, ponds with high noise levels under the flight-path of Inverness airport were readily used, as were sites where shooting takes place, suggesting that noise in itself may not be problematic. As expected, macroinvertebrate richness was positively related to population persistence. Significant relationships were found with shore habitat within 1 m of the water's edge; while high coverage of grass and moss was associated with persistence, high density of trees was linked to disappearance. Together with the lack of a significant impact of shading, this may suggest that, at least at high latitudes, shore vegetation is important for shading as well as for foraging and shelter. Other factors showing a positive relationship with persistence were adjacent mixed *Pinus sylvestris* - *Betula* woodland (within 500 m of the pond), and the likelihood of pond drying, agreeing with the statistical inferences based on presence data. Many of these biotic and abiotic factors are amenable to conservation intervention which enhance the resilience of populations to stochastic events and adverse effects, for example by planting appropriate tree species or taking steps to manage desiccation frequency.

Conclusion

Triturus cristatus habitat requirements at the edge of its range differ from those at the core. Most of the habitat characteristics which we found to be significant may be managed (mainly those associated with the quality of the pond and surrounding habitat), and the results are already being used to inform the design of conservation interventions. The results of this study provide practical criteria for managing existing ponds and creating new ones, thus mitigating risks to the conservation of *T. cristatus* peripheral populations. We are currently working with landowners to create new ponds within the dispersal range of existing ponds and within habitat types most strongly associated with presence and persistence. The management of existing ponds focuses on the maintenance and enhancement of features associated with *T. cristatus* persistence.

Acknowledgements

This project was carried out under licence 29609 by Scottish Natural Heritage. The authors would like to acknowledge all people who helped us during the field and the desk work, especially Karen Frake, who prepared the maps. We also want to thank Scottish Natural Heritage for the use of a pool car and fuel, and our families for their continuing support. Most importantly, we would like to thank the landowners of the survey sites for granting access and for their enthusiasm for the protection of these animals. We thank the three anonymous reviewers and Prof Chris Joyce by their valuable comments which helped to improve the manuscript.

Compliance with ethical standards

Conflict of Interest The authors declare that they have no conflict of interest.

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Tables:

Table 1

Selected predictor variables used for the statistical analyses. Abbreviations are given in brackets (upper case for numerical variables and lower case for categorical variables). Detailed information about all variables is given in Online Resource 1.

Variable type	Variable name	Description
Site features	Drying (DRYING)[a, b]	Number of years in 10 when the pond dries up, log(x+1) transformed
Macroinvertebrates	Macroinvertebrate richness (MINVRICH)[a, b]	Number of defined macroinvertebrate taxa present in the pond (see Online Resource 1)
Potential predators	Fish presence (Fish)[a, b]	Binary factor determined by fish presence in the pond
Aquatic vegetation	Aquatic macrophytes (MACROPH)[a]	% coverage of the pond occupied by submerged and emergent macrophytes except aquatic mosses and *Lemna* sp.
Bank slope	Slightly sloping bank (LITTLE)[a]	% of pond perimeter with slightly sloping banks, log(x+1) transformed
Pond substrate	Substrate organic mud (ORMUD)[a]	% of pond substrate comprising organic mud (mainly decaying stem and leaf debris)
Shore habitat	Shore moss coverage (MOSS)[a, b]	% of moss coverage of the pond shore, log(x+1) transformed
	Shore grass coverage (GRASS)[b]	% of grass coverage of the pond shore, log(x+1) transformed
	Shore tree coverage (TREE_WOOD)[b]	% of tree coverage of the pond shore, log(x+1) transformed
Terrestrial habitat	Adjacent mixed woodland (MIXEDWOOD)[a, b]	% of adjacent terrestrial habitat comprising mixed *Pinus sylvestris - Betula* woodland, log(x+1) transformed
	Terrestrial habitat diversity (TERRSHAN)[a]	Shannon diversity index of adjacent terrestrial habitats
Stressor structure	PC2 for stressor structure (STRESPC2)[b]	Second principal component for all anthropogenic stressors identified. This is negatively correlated with proportion of pond surrounded by roads.
Geology categories	Boulder clay over middle old red sandstone (BoulderClayC2)[a]	Binary factor indicating dominant geological category Boulder clay over middle old red sandstone in the pond area
	Sand and gravel over middle old red sandstone (SandGravelC2)[a]	Binary factor indicating dominant geological category Sand and Gravel over middle old red sandstone in the pond area
Soil categories	Humus-rich iron podzols (Soil97)[1]	Binary factor indicating dominant humus-rich iron podzols around the pond

[a] variables selected for the *T. cristatus* occurrence analyses

[b] variables selected for the *T. cristatus* persistence analysis.

Figure captions

Fig. 1 *Triturus cristatus* records in the Scottish Highlands since 1990. Symbols represent presence (light grey), absence (white) and disappearance (dark grey) as determined by field surveys (2010-2015) and previous records.

Fig. 2 Distance PCA for (a) the 12 selected variables for the occurrence analyses and for (b) the eight selected variables for the persistence analyses. Colour of symbols indicates presence (light grey), absence (white) and disappearance (dark grey) of *T. cristatus*. Circles indicate fish absence and diamonds indicate fish presence. Categorical variables were used directly as dummy (1/0) variables. Abbreviations used are: number of years in 10 when the pond dries up (DRYING), fish presence (Fish), macroinvertebrate richness (MINVRICH), coverage of the pond occupied by submerged and emergent macrophytes except aquatic mosses and *Lemna* sp. (MACROPH), pond substrate comprising organic mud (ORMUD), proportion of slightly sloping banks (LITTLE), moss coverage of the pond shore (MOSS), coverage of adjacent mixed *Pinus sylvestris* - *Betula* woodland (MIXEDWOOD), adjacent terrestrial habitat diversity (TERRSHAN), dominant geological category Sand and Gravel over middle old red sandstone in the pond area (SandGravelC2), dominant geological category Boulder Clay over middle old red sandstone in the pond area (BoulderClayC2), dominant humus-rich iron podzols around the pond (Soil97), grass coverage of the pond shore (GRASS), tree coverage of the pond shore (TREE_WOOD) and second principal component for all anthropogenic stressor types (STRESPC2). Note that high values of STRESPC2 are strongly correlated with low presence of adjacent roads (Table 9 in Online Resource 1). Abbreviations of numerical variables are written with upper case and abbreviations of categorical dummy transformed variables are written with lower case.

Fig. 3 Importance of variables based on the non-parametric random forests for *T. cristatus* (a) occurrence and (b) persistence analyses. Area under the curve (AUC) was used to generate the Variable Importance Measure (VIM) for each variable. Abbreviations of numerical variables are written with upper case and abbreviations of categorical dummy transformed variables are written with lower case. Abbreviations are: number of years in 10 when the pond dries up (DRYING), fish presence (Fish), macroinvertebrate richness (MINVRICH), coverage of the pond occupied by submerged and emergent macrophytes except aquatic mosses and *Lemna* sp. (MACROPH), pond substrate coverage comprising organic mud (ORMUD), proportion of slightly sloping banks (LITTLE), moss coverage of the pond shore (MOSS), coverage of adjacent mixed *Pinus sylvestris* - *Betula* woodland (MIXEDWOOD), adjacent terrestrial habitat diversity (TERRSHAN), dominant geological category Sand and Gravel over middle old red sandstone in the pond area (SandGravelC2), dominant geological category Boulder clay over middle old red sandstone in the pond area (BoulderClayC2), dominant humus-rich iron podzols around the pond (Soil97), grass coverage of the pond shore (GRASS), tree coverage of the pond shore (TREE_WOOD) and second principal component for all anthropogenic stressor types (STRESPC2). Note that STRESPC2 is strongly correlated with low presence of adjacent roads (Table 9 in Online Resource 1).

Figures

Fig. 1

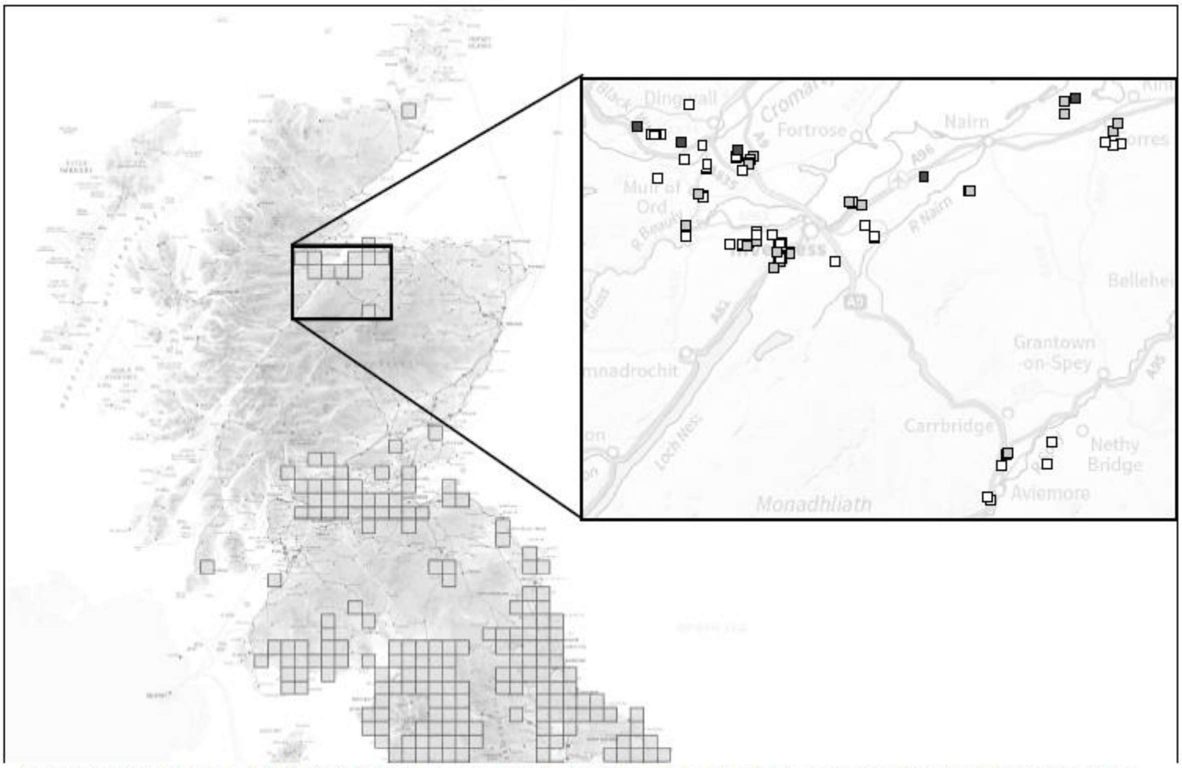
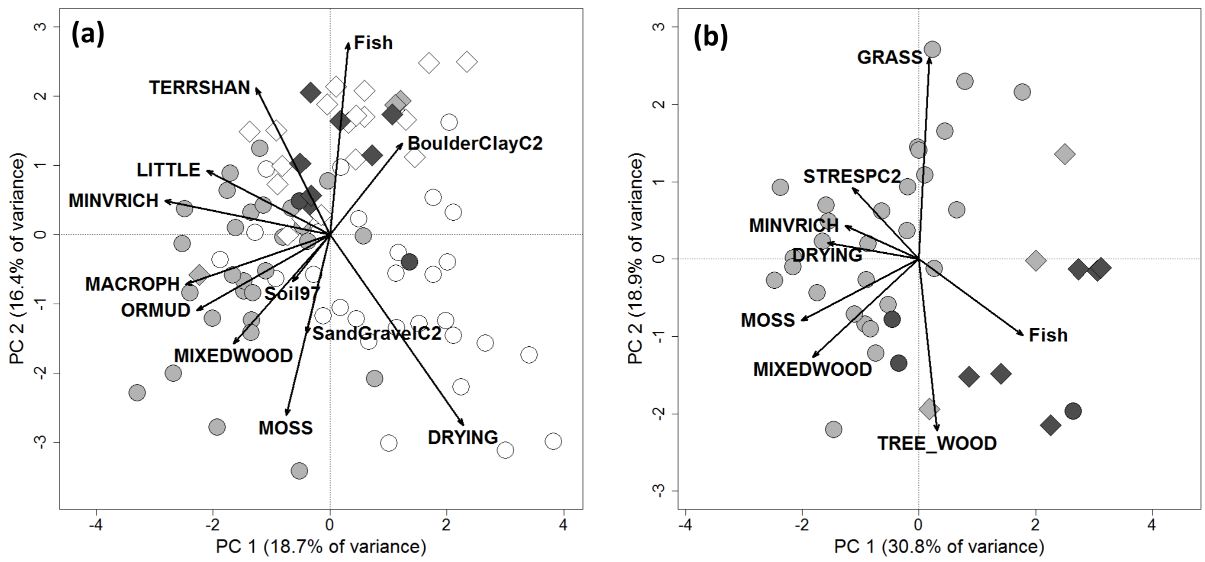
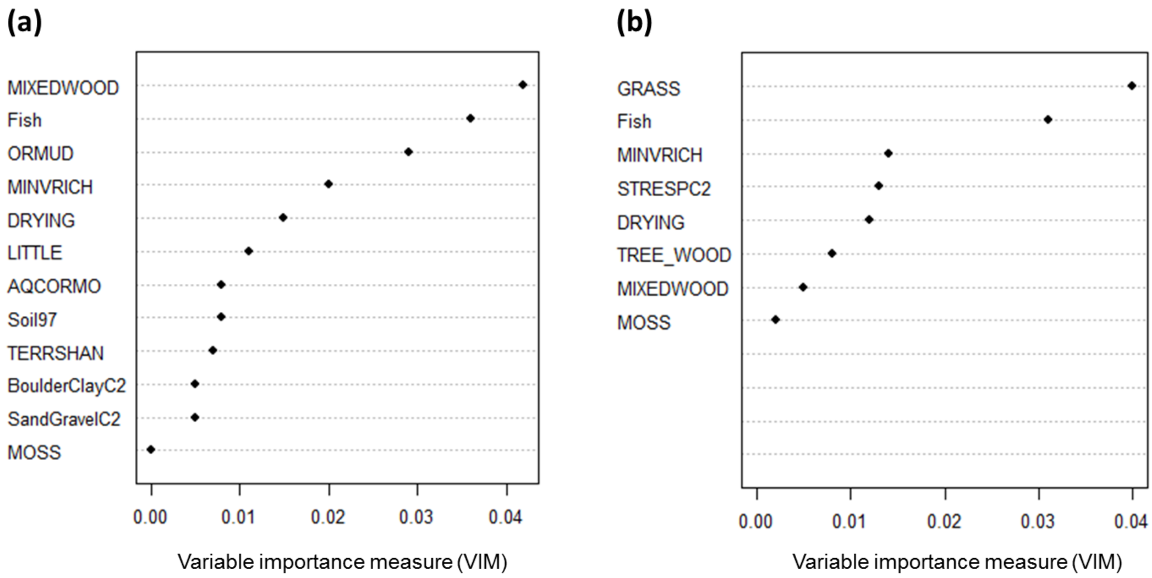


Fig. 2



(a)
Fish
TERRSHAN
BoulderClayC2
LITTLE
MINVRICH
MACROPH
ORMUD
Soil97
SandGravelC2
MIXEDWOOD
MOSS
DRYING
PC 2 (16.4% of variance)
PC 1 (18.7% of variance)
(b)
GRASS
STRESPC2
MINVRICH
DRYING
MOSS
MIXEDWOOD
Fish
TREE_WOOD
PC 2 (18.9% of variance)
PC 1 (30.8% of variance)

Fig. 3



(a)
MIXEDWOOD
Fish
ORMUD
MINVRICH
DRYING
LITTLE
AQCORMO
Soil97
TERRSHAN
BoulderClayC2
SandGravelC2
MOSS
0.00
0.01
0.02
0.03
0.04
Variable importance measure (VIM)
(b)
GRASS
Fish
MINVRICH
STRESPC2
DRYING
TREE_WOOD
MIXEDWOOD
MOSS
0.00
0.01
0.02
0.03
0.04
Variable importance measure (VIM)

Online Resource 1

Habitat requirements and conservation needs of peripheral populations: The case of the great crested newt (*Triturus cristatus*) in the Scottish Highlands.

Hydrobiologia

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Supplementary materials and methods

Detailed description of habitat variables

The set of predictor variables generated encompasses 129 habitat features, 88 gathered during field work and 41 generated through desk study after the field sampling period. The whole perimeter of the shore was sampled for ponds smaller than 0.3 ha. For larger ponds, at least 300 m of shore line, covering all habitats present was sampled. The quantification of the habitat features was estimated from the individual measurements of the surveyors present. The different habitat classifications and categories were adapted from the field forms of previous surveys of Scottish ponds commissioned by Scottish Natural Heritage (e.g. Alexander 1997) and from previous studies focussed on the relation between habitat characteristics and the distribution of *T. cristatus* or other amphibians (Beebee 1985; Gustafson et al. 2009; Gustafson et al. 2011; Knapp 2005; Maletzky et al. 2007; Miró 2016; Pilliod et al. 2010; Skei et al. 2006; Sztatecsny et al. 2004).

The topographical features of the ponds were characterized using the following fifteen variables. Altitude (m), surface area (m^2), shore perimeter (m), and geographical coordinates UTM X and Y (m) of the studied ponds were obtained from a GIS using 1:25 000 maps from the British mapping agency Ordnance Survey. Pond density, defined as ponds/km^2 within a 1km radius (ARG-UK 2010; Oldham et al. 2000), and pond age, defined as the year when the pond was formed (for those not present on the oldest available maps), were also obtained from recent and historical Ordnance Survey maps, going back to c. 1870. Water conductivity (µS/cm, corrected to 20ºC) and water pH were gathered during field survey with handheld meters WTW Cond 340i and Jenway 350 pH meter respectively. Field oxygen concentration (mg/l) and oxygen saturation (%) were sampled using a handheld meter OxyGuard Handy MkII. Water transparency was estimated using a semi-quantitative scale from 0 (opaque) to 5 (completely transparent). Maximum depth (m) was obtained from previous surveys commissioned by Scottish Natural Heritage or was estimated by the surveyors. Drying (pond permanence) and pond shading (ARG-UK 2010; Oldham et al. 2000) were also surveyed. Drying, defined as number of years in 10 when the pond dries up, was given by the owners or estimated by the surveyors based on their experience in the area. Pond shading was evaluated as the % of the pond surface affected by shade during the breeding season.

Macroinvertebrate community was assessed by dip-netting the different habitats found in the pond in proportion to their coverage. The entire perimeter was swept for ponds <100m^2, at least 50 sweeps were made in ponds >100 m^2 and at least 100 were made in ponds >1 000m^2. Eleven macroinvertebrate taxa or general groups were identified in the field and returned to the pond. After finishing the sampling of each pond, the surveyors estimated the abundance of each taxon using a semi-quantitative scale (0-5). The 11 macroinvertebrate taxa identified were: adult pond skaters (Family Gerridae), adult back-swimmers (Family Notonectidae), adults and larvae of black and brown beetles (Order Coleoptera), larvae of dragonflies (Order Odonata), mayflies (Order Ephemeroptera), caddisflies (Order Trichoptera) and gnats (Order Diptera), and adult leeches (Subclass Hirudinea), molluscs (Phylum Mollusca) and large crustaceans (>0.5cm) such as *Gammarus* sp. Two more variables were calculated subsequently: macroinvertebrate richness (total number of previous described taxa present) and macroinvertebrate diversity (Shannon diversity index for the macroinvertebrate community of each pond).

The presence of fish and waterfowl predators was also evaluated. Fish presence was determined from interviews with local fishermen, visual surveys and sampling effort through funnel traps, mesh nets and electro-fishing when necessary. The main fish species found were three-spined stickleback (*Gasterosteus aculeatus*), brown trout (*Salmo trutta*) and rainbow trout (*Onorhynchus mykiss*). All three fish species detected were usually present in high densities and are considered predators of *T. cristatus*, in addition to negative indirect impacts on embryos viability and habitat use (Hartel et al. 2007; Jarvis 2010; Winandy et al. 2015). Waterfowl abundance was assessed using a semi-quantitative scale (0-5) on the basis of bird observations during field work and on surveyors' previous sampling experience in the area. The bird species encountered (in order of frequency) were mallard (*Anas platyrhynchos*), tufted duck (*Aythya fuligula*), Slavonian grebe (*Podiceps auritus*), grey heron (*Ardea cinerea*), European coot (*Fulica atra*), moorhen (*Gallinula chloropus*), little grebe (*Tachybaptus ruficollis*), mute swan (*Cygnus olor*), greylag goose (*Anser anser*) and common teal (*Anas crecca*).

Aquatic vegetation was assessed by estimating the percent cover of different types of macrophytes. The categories used were: aquatic mosses, *Lemna* sp., filamentous green algae, hydrophytes (floating-leaved and submerged macrophytes, except aquatic mosses and *Lemna* sp.), helophytes (emergent macrophytes), and two combinations, all macrophytes except *Lemna* sp. (hydrophytes + helophytes + aquatic mosses) and all macrophytes except aquatic mosses and *Lemna* sp. (hydrophytes + helophytes). During the field surveys the percentage of the pond water column with vegetation and the percentage of coverage by soft-leaved macrophytes (which are easier for *T. cristatus* to fold and lay eggs on) were also estimated.

Bank slope was estimated visually and expressed as % coverage for the categories: shallow (<10 cm deep), flat (0-10º), slightly sloping (20-30º), moderate sloping (roughly 40º), quite slope (50-60º), very slope (70-80º) and vertical bank (roughly 90º).

Pond substrate was evaluated by estimating the % coverage of the following categories: boulders (>30 cm diameter), stones (5-30 cm), gravel (4-50 mm), sand (0.1-4 mm), silt (< 0.1 mm), coarse woody debris, organic mud (mainly decaying stem and leaf debris), peat and artificial substrate.

Shore habitat was characterized by estimating its composition from the water's edge to 1 m onto the surrounding land, as % coverage of the following categories: boulders (>30 cm), stones (<30 cm), moss, grass, scrub, trees and artificial embankment.

The relief of the surrounding land was estimated using a semi-quantitative scale from 0 (flat) to 5 (sheer). Adjacent terrestrial habitat was characterized by estimating its composition from the water's edge to approximately 500 m into the surrounding land as % coverage of the following categories (EUNIS alphanumeric code in brackets, European Environment Agency 2014): rocks, intensive unmixed crops (I1.1), cultivated areas of gardens and parks (I2), mesic grassland (E2), Temperate thickets and scrub (F3.1), mixed *Pinus sylvestris* - *Betula* woodland (G4.4), broadleaved deciduous woodland (G1), *Pinus sylvestris* woodland (G3.4), highly artificial coniferous plantations (G3.F), recently felled areas (G5.8), surface running waters (C2), mires, bogs and fens (D), surface standing waters (C1), low density buildings (J2), residential buildings of village and urban peripheries (J1.2), road networks (J4.2) – subdivided into sand/forestry road and asphalt road. Two more variables were calculated subsequently: number of terrestrial habitats (the total number of categories presents in the surrounding area) and terrestrial habitat diversity (Shannon diversity index).

The effect of anthropogenic activities was assessed by estimating the influence of several stressors on the pond, and within 500 m of the pond. A semi-quantitative scale (0-5) was used to evaluate the importance of each stressor: water abstraction, dam/impoundment, artificial water-level fluctuation, sewage inflow, agricultural pollution, grazing, edge trampling, commercial forestry, shore fishing, boat fishing, shooting, human frequentation, noise, levelling of land and aggregate extraction. In the case of shooting, we considered any sort of game shooting, primarily based on interviews with landowners and corroborated by the presence of any evidence such as hunter's shelters or gun cartridges. To reduce the dimensionality of the anthropogenic effect, we subjected the stressor structure to a principal component analyses (e.g. Knapp 2005), where axes 1 and 2 explained a substantial amount of the total variation (axis 1: 40.3%; axis 2: 17.7%), hence the scores were used as the independent variable representing the principal components 1 and 2 of the stressor structure. Principal component 1 was positively correlated to human frequentation, and negatively correlated to agricultural pollution, grazing, edge trampling, shooting, fences and noise. Principal component 2 was positively correlated to shooting and negatively correlated to roads and noise (Table 8). Since most of the noise came from roads, the stressor noise was also strongly positively correlated with roads (Spearman's rho = 0.37, P = 0.009).

Using geological and soil maps (Institute of Geological Sciences 1973; Macaulay Institute for Soil Research 1981) we generated both categorical variables descriptive of the underlying geological category in the pond area and of the soil category around the pond.

The geological categories identified were:

- Basal breccia and Conglomerate,
- Blown sand,
- Boulder clay over middle old red sandstone,
- Boulder clay/Undifferentiated schist,
- Fluvio-glacial deposits over middle old red sandstone C2,
- Moraine drift over middle old red sandstone,
- Present terrace 1,
- Present terrace 2,
- Raised beach 1,
- Raised beach 2,
- Raised beach 3,
- Sand and gravel,
- Sand and gravel over middle old red sandstone C2,
- Sand and gravel over upper old red sandstone C2,
- Sand and gravel/Undifferentiated schist
- Undifferentiated schist.

The soil categories identified were:

- alluvial soils-1,
- peaty podzols, humus-iron podzols; some peaty gleys and rankers-28,
- rankers, peaty podzols; some humus iron podzols and peaty gleys-30,
- brown forest soils-71,
- Humus iron podzols; some gleys -97,
- Humus iron podzols; some peaty gleys and humic gleys-100,
- Humus iron podzols; some brown forest soils and gleys-282,
- Regosols; some gleys-380,
- Noncalcareous gleys; some peaty gleys and peat-405,
- Humus iron podzols; some noncalcareous gleys-406,
- Noncalcareous gleys-421.
- Humus iron podzols; some peaty podzols and gleys-425,
- Humus iron podzols; some gleys and peaty podzols-454

Both geology and soil categorical variables were used as dummy (1/0) transformed variables.

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Supplementary tables

Table 2 Variance Inflation Factors (VIF) of the 12 selected variables for the *T. cristatus* occurrence analyses and geographical UTM coordinates. Drying (DRYING), slightly sloping bank (LITTLE), shore moss (MOSS) and mixed *Pinus sylvestris* - *Betula* woodland (MIXEDWOOD) were previously log(x+1) transformed. Abbreviations are given in brackets and are written with upper case for numerical variables and with lower case for categorical variables.

Variable type	Variable name	VIF value
Site features	Drying (DRYING)	2.583
Macroinvertebrates	Macroinvertebrate richness (MINVRICH)	1.755
Potential predators	Fish presence (Fish)	1.952
Aquatic vegetation	Aquatic macrophytes except aquatic mosses and *Lemna* sp. (MACROPH)	1.526
Bank sloping	Slightly sloping bank (LITTLE)	1.693
Substrate habitat	Substrate of organic mud (ORMUD)	1.441
Shore habitat	Shore moss coverage (MOSS)	1.476
Terrestrial habitat	Adjacent mixed *Pinus sylvestris* - *Betula* woodland (MIXEDWOOD)	1.406
	Terrestrial habitat diversity (TERRSHAN)	1.586
Geology categories	Boulder clay over middle old red sandstone (BoulderClayC2)	1.219
	Sand and gravel over middle old red sandstone (SandGravelC2)	1.641
Soil categories	Humus-rich iron podzols (Soil97)	1.925
Geographical	UTM x coordinate (UTMx)	1.868
	UTM y coordinate (UTMy)	1.301

Table 3 Variance Inflation Factors (VIF) of the eight selected variables for the *T. cristatus* persistence analyses and geographical UTM coordinates. Drying (DRYING), shore moss (MOSS) and mixed *Pinus sylvestris* - *Betula* woodland (MIXEDWOOD), shore grass (GRASS) and shore tree coverage (TREE_WOOD) were previously log(x+1) transformed. Abbreviations are given in brackets and are written with upper case for numerical variables and with lower case for categorical variables. Note that STRESPC2 is negatively correlated with the presence of adjacent roads (table 9).

Variable type	**Variable name**	**VIF value**
Site features	Drying (DRYING)	1.833
Macroinvertebrates	Macroinvertebrate richness (MINVRICH)	1.446
Potential predators	Fish presence (Fish)	1.842
Shore habitat	Shore moss coverage (MOSS)	2.056
	Shore grass coverage (GRASS)	1.752
	Shore tree coverage (TREE_WOOD)	1.285
Terrestrial habitat	Adjacent mixed *Pinus sylvestris* - *Betula* woodland (MIXEDWOOD)	2.146
Stressor structure	PC2 for stressor structure (STRESPC2)	1.439
Geographical	UTM x coordinate (UTMx)	1.211
	UTM y coordinate (UTMy)	1.379

Table 4 Pearson correlation matrix of numerical selected variables for *T. cristatus* occurrence analyses and geographical UTM coordinates. Drying (DRYING), slightly sloping bank (LITTLE), shore moss (MOSS) and mixed *Pinus sylvestris* - *Betula* woodland (MIXEDWOOD) were previously log(x+1) transformed. The other abbreviations indicate: macroinvertebrate richness (MINVRICH), coverage of the pond occupied by submerged and emergent macrophytes except aquatic mosses and *Lemna* sp. (MACROPH), substrate pond coverage of organic mud (ORMUD) and adjacent terrestrial habitat diversity (TERRSHAN). *denotes correlation significantly different from zero ($P < 0.05$).

	ORMUD	LITTLE	MINVRICH	MACROPH	MOSS	MIXEDWOOD	TERRSHAN	UTMx	UTMy
DRYING	-0.070	-0.512*	-0.380*	-0.080	0.226*	0.017	-0.328*	-0.279*	0.122
ORMUD		0.138	0.246*	0.364*	0.329*	0.202	0.095	0.058	0.050
LITTLE			0.289*	0.071	-0.129	0.029	0.305*	0.314*	-0.018
MINVRICH				0.409*	0.163	0.260*	0.170	0.119	-0.107
MACROPH					0.289*	0.187	-0.043	0.250*	-0.087
MOSS						0.354*	-0.058	-0.025	0.056
MIXEDWOOD							0.128	0.217*	0.022
TERRSHAN								-0.205	0.225*
UTMx									-0.317*

Table 5 Spearman correlation matrix of numerical and categorical selected variables for *T. cristatus* occurrence analyses and geographical coordinates. The binary factors sand and gravel over middle old red sandstone (SandGravelC2), boulder clay over middle old red sandstone (BoulderClayC2), humus-rich iron podzols (Soil97) and fish presence (Fish), were used directly as dummy (1/0) variables. The other abbreviations indicate: number of years in 10 when the pond dries up (DRYING), macroinvertebrate richness (MINVRICH), coverage of the pond occupied by submerged and emergent macrophytes except aquatic mosses and *Lemna* sp. (MACROPH), substrate pond coverage of organic mud (ORMUD), slightly sloping banks (LITTLE), moss coverage of the pond shore (MOSS), coverage of adjacent mixed *Pinus sylvestris* - *Betula* woodland (MIXEDWOOD) and adjacent terrestrial habitat diversity (TERRSHAN). *denotes correlation significantly different from zero ($P < 0.05$).

	ORMUD	LITTLE	SandGravelC2	BoulderClayC2	Soil97	Fish	MINVRICH	MACROPH	MOSS	MIXEDWOOD	TERRSHAN	UTMx	UTMy
DRYING	0.003	-0.433*	-0.016	-0.043	-0.130	-0.552*	-0.241*	-0.020	0.259*	0.101	-0.235*	-0.308*	0.110
ORMUD		0.134	-0.122	-0.222*	0.039	-0.203	0.259*	0.422*	0.320*	0.198	0.065	-0.025	0.150
LITTLE			0.155	-0.119	0.311*	0.155	0.249*	0.081	-0.117	0.019	0.241*	0.354*	-0.023
SandGravelC2				-0.180	0.539*	-0.193	0.030	-0.103	-0.067	-0.076	-0.219*	0.291*	-0.044
BoulderClayC2					-0.208	0.026	-0.073	-0.124	-0.142	-0.142	-0.058	-0.021	-0.066
Soil97						-0.078	-0.045	-0.161	-0.170	-0.111	-0.080	0.355*	-0.033
Fish							-0.159	-0.065	-0.318*	-0.164	-0.005	0.149	-0.002
MINVRICH								0.412*	0.154	0.222*	0.119	0.092	0.008
MACROPH									0.302*	0.214*	-0.055	0.122	0.174
MOSS										0.352*	-0.031	-0.097	0.111
MIXEDWOOD											0.104	0.115	0.124
TERRSHAN												-0.202	0.082
UTMx													-0.300*

Table 6 Pearson correlation matrix of numerical selected variables for *T. cristatus* persistence analyses and geographical coordinates. Drying (DRYING), shore moss (MOSS) and mixed *Pinus sylvestris* - *Betula* woodland (MIXEDWOOD), shore grass (GRASS) and shore tree coverage (TREE_WOOD) were previously log(x+1) transformed. The other abbreviations are: macroinvertebrate richness (MINVRICH) and second principal component for all anthropogenic stressor types (STRESPC2). *denotes correlation significantly different from zero ($P < 0.05$). Note that STRESPC2 is inversely correlated with the presence of adjacent roads (table 9).

	MINVRICH	MOSS	GRASS	TREE_WOOD	MIXEDWOOD	STRESPC2	UTMx	UTMy
DRYING	-0.009	0.391*	0.182	-0.070	0.469*	0.063	0.106	-0.213
MINVRICH		0.297	0.034	0.025	0.151	0.207	0.025	-0.023
MOSS			-0.314*	-0.104	0.495*	0.208	0.047	0.056
GRASS				-0.247	-0.199	0.160	-0.057	-0.305
TREE_WOOD					0.182	-0.132	0.150	0.088
MIXEDWOOD						0.290	0.304	-0.036
STRESPC2							-0.101	0.156
UTMx								-0.013

Table 7 Spearman correlation matrix of numerical and categorical selected variables for *T. cristatus* persistence analyses and geographical coordinates. The binary factor fish presence (Fish) was used directly as dummy (1/0) variable. The other abbreviations are: drying (DRYING), shore moss (MOSS) and mixed *Pinus sylvestris* - *Betula* woodland (MIXEDWOOD), shore grass (GRASS) and shore tree coverage (TREE_WOOD) were previously log(x+1) transformed. The other abbreviations are: macroinvertebrate richness (MINVRICH) and second principal component for all anthropogenic stressor types (STRESPC2). *denotes correlation significantly different from zero ($P < 0.05$). Note that STRESPC2 is negatively correlated with the presence of adjacent roads (table 9).

	Fish	MINVRICH	MOSS	GRASS	TREE_WOOD	MIXEDWOOD	STRESPC2	UTMx	UTMy
DRYING	-0.389*	0.061	0.366*	0.054	-0.009	0.496*	0.064	0.033	-0.056
Fish		-0.461*	-0.402*	-0.088	0.193	-0.254	-0.184	0.029	0.345*
MINVRICH			0.240	0.018	-0.009	0.087	0.145	0.109	0.022
MOSS				-0.389*	-0.152	0.486*	0.194	-0.019	0.115
GRASS					-0.404	-0.263	0.126	0.017	-0.376*
TREE_WOOD						0.157	-0.108	0.034	0.142
MIXEDWOOD							0.205	0.210	0.130
STRESPC2								-0.142	0.016
UTMx									-0.018

Table 8 Importance of variables within the random forest classification analysis for *T. cristatus* occurrence. Variables are ordered from higher to lower importance, expressed as Variable Importance Measure (VIM) and computed by the area under the curve (AUC) algorithm. Abbreviations are given in brackets and are written with upper case for numerical variables and with lower case for categorical variables.

Variable name	Variable importance measure (VIM)
Adjacent mixed *Pinus sylvestris* - *Betula* woodland (MIXEDWOOD)	0.042
Fish presence (Fish)	0.036
Substrate of organic mud (ORMUD)	0.029
Macroinvertebrate richness (MINVRICH)	0.020
Drying (DRYING)	0.015
Slightly sloping bank (LITTLE)	0.011
Aquatic macrophytes except aquatic mosses and *Lemna* sp. (MACROPH)	0.008
Humus-rich iron podzols (Soil97)	0.008
Terrestrial habitat diversity (TERRSHAN)	0.007
Boulder clay over middle old red sandstone (BoulderClayC2)	0.005
Sand and gravel over middle old red sandstone (SandGravelC2)	0.005
Shore moss coverage (MOSS)	0

Table 9 Spearman's correlations among the stressor structure constituents with the principal component axis 2, for the data subset used in the *T. cristatus* persistence analyses. The significant terms (P< 0.05) are highlighted in bold type.

Stressor structure	Spearman's Rho	Signification
Dam-impoundment	0.229	0.155
Artificial water-level fluctuation	-0.062	0.702
Agricultural pollution	-0.016	0.922
Grazing	0.204	0.207
Edge trampling	0.295	0.065
Forestry exploitation	0.115	0.481
Shore fishing	0.008	0.960
Boat fishing	0.144	0.375
Shooting	**0.352**	**0.026**
Surrounding roads	**-0.711**	**<0.001**
Surrounding fences	0.027	0.869
Human frequentation	-0.179	0.268
Noise	**-0.580**	**<0.001**
Levelling of land	-0.271	0.091
Aggregate extraction	-0.214	0.185

Table 10 Importance of variables within the random forest classification analysis for *T. cristatus* persistence. Variables are ordered from higher to lower importance, expressed as Variable Importance Measure (VIM) and computed by the area under the curve (AUC) algorithm. Abbreviations are given in brackets and are written with upper case for numerical variables and with lower case for categorical variables. Note that PC2 for stressor structure (STRESPC2) is inversely correlated with the presence of adjacent roads (table 9).

Variable name	Variable importance measure (VIM)
Shore grass (GRASS)	0.040
Fish presence (Fish)	0.031
Macroinvertebrate richness (MINVRICH)	0.014
PC2 for stressor structure (STRESPC2)	0.013
Drying (DRYING)	0.012
Shore tree coverage (TREE_WOOD)	0.008
Adjacent mixed *Pinus sylvestris* - *Betula* woodland (MIXEDWOOD)	0.005
Shore moss coverage (MOSS)	0.002

Supplementary figures

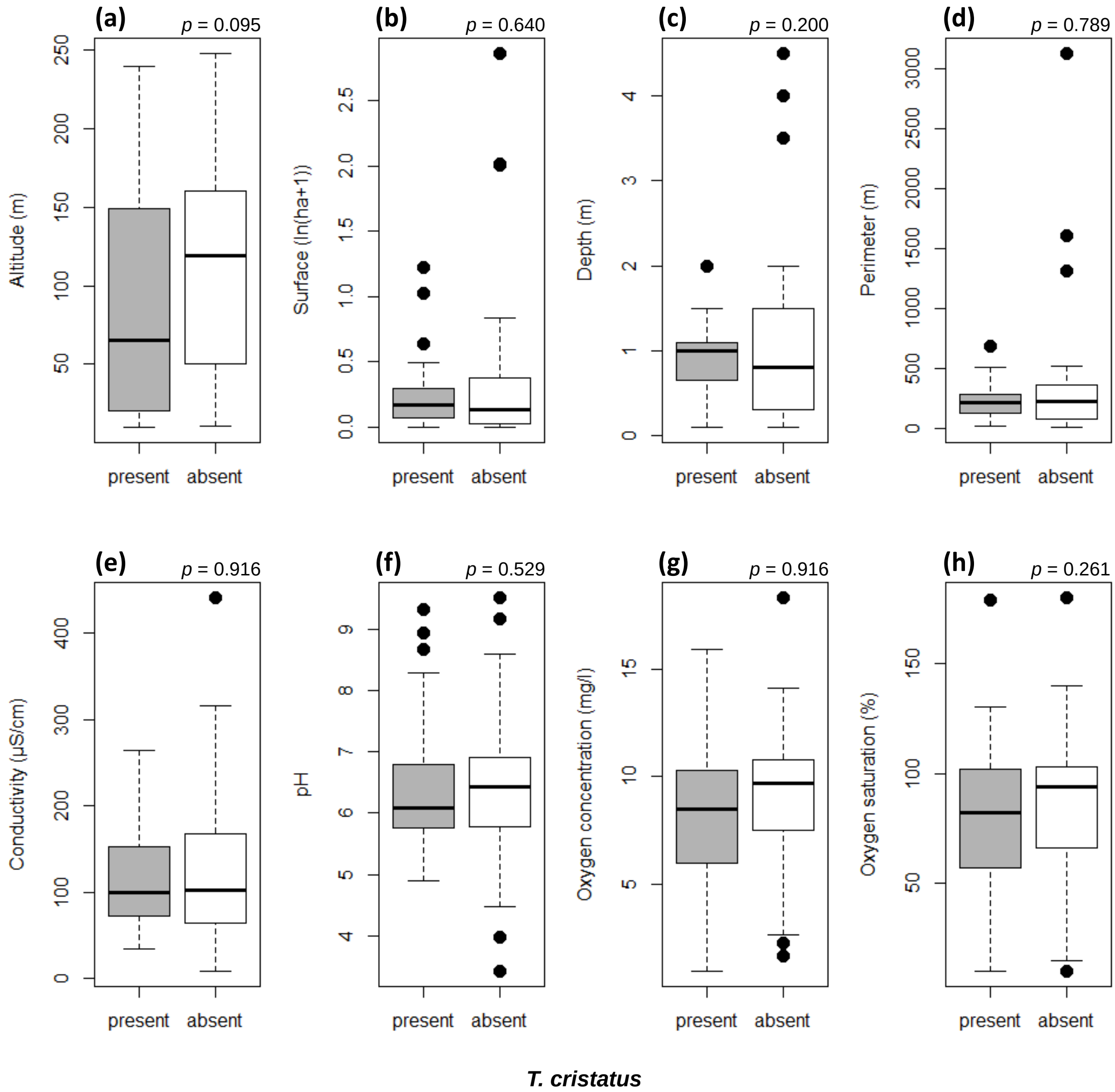
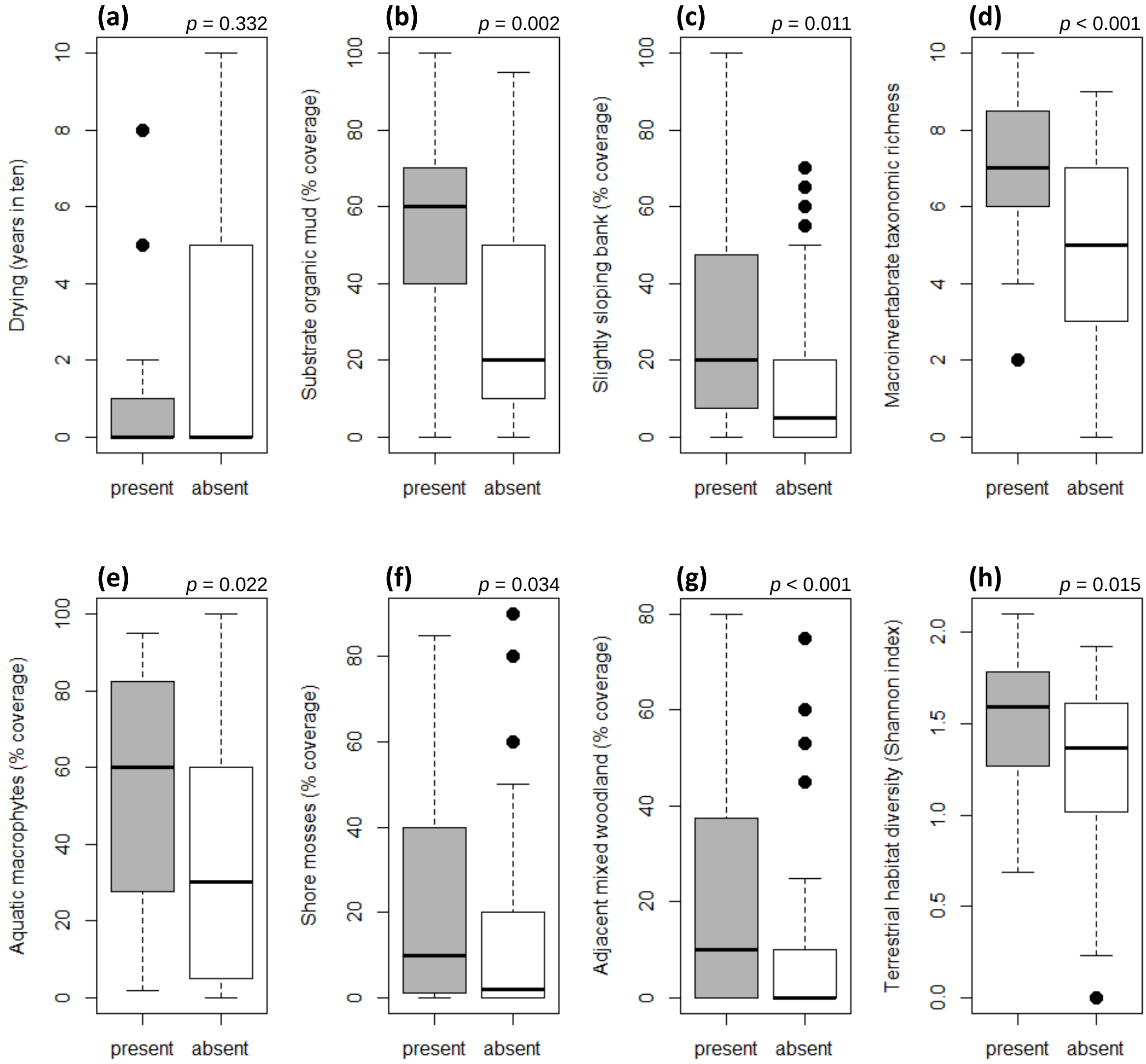


Fig. 4 Box plots of general characteristics of the ponds studied, showing (a) altitude, (b) surface area, (c) maximum depth, (d) shore perimeter, (e) conductivity, (f) pH, (g) oxygen concentration and (h) oxygen saturation of the ponds with presence (n = 31) and absence (n = 57) of *T. cristatus*. The line within each box marks the median, the bottom and top of each box indicate the 25th and 75th percentiles, the whiskers below and above each box indicate the 10th and 90th percentiles, and the points above and below the whiskers indicate the 5th and 95th percentiles. The *P* values of the U Mann-Whitney tests are given on the top of the boxes.


(a)
p = 0.332
Drying (years in ten)
(b)
p = 0.002
Substrate organic mud (% coverage)
(c)
p = 0.011
Slightly sloping bank (% coverage)
(d)
p < 0.001
Macroinvertabrate taxonomic richness
(e)
p = 0.022
Aquatic macrophytes (% coverage)
(f)
p = 0.034
Shore mosses (% coverage)
(g)
p < 0.001
Adjacent mixed woodland (% coverage)
(h)
p = 0.015
Terrestrial habitat diversity (Shannon index)
present
absent

T. cristatus

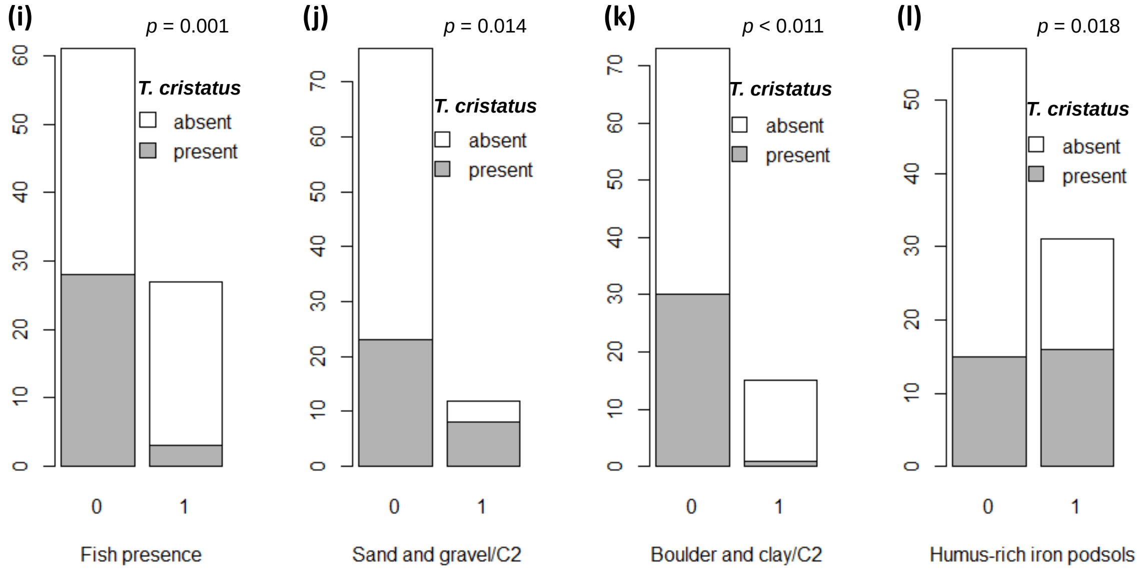
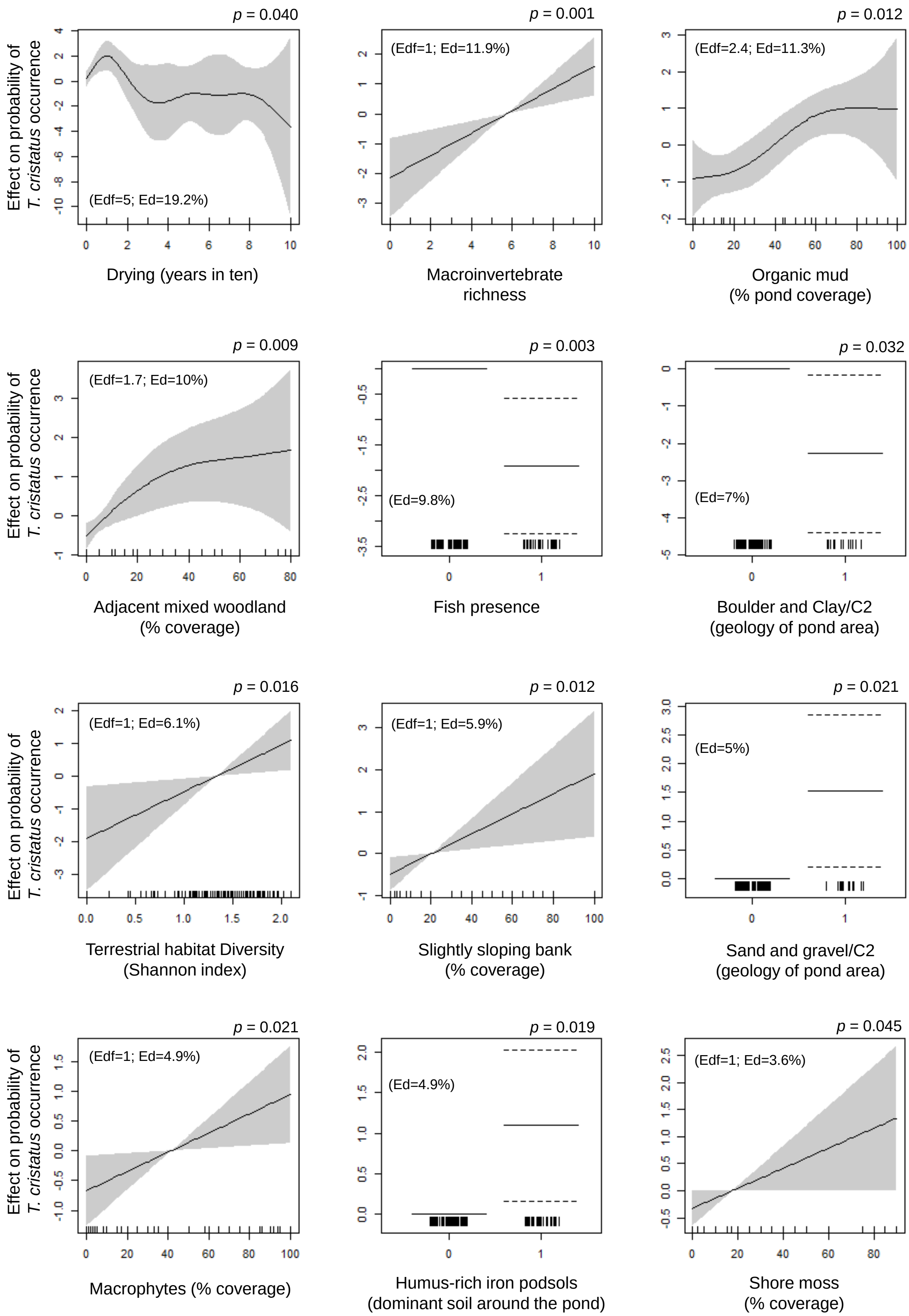


Fig. 5 Box plots and bar charts corresponding to the 12 selected variables and factors for *T. cristatus* occurrence analyses, showing (a) years in 10 when the pond dries up, (b) substrate organic mud, (c) slightly sloping bank, (d) macroinvertebrate taxonomic richness, (e) aquatic macrophytes except aquatic mosses and *Lemna* sp., (f) shore moss, (g) adjacent mixed *Pinus sylvestris* - *Betula* woodland, (h) terrestrial habitat diversity, (i) fish presence, (j) sand and gravel over middle old red sandstone (k) boulder clay over middle old red sandstone and (l) humus-rich iron podzols of the ponds with presence (n = 31) and absence (n = 57) of *T. cristatus*. The line within each box marks the median, the bottom and top of each box indicate the 25th and 75th percentiles, the whiskers below and above each box indicate the 10th and 90th percentiles, and the points above and below the whiskers indicate the 5th and 95th percentiles. The *P* values of the U Mann-Whitney and χ^2 tests are given on the top of the boxes for numerical and categorical variables respectively.



Effect on probability of
T. cristatus occurrence
p = 0.040
(Edf=5; Ed=19.2%)
Drying (years in ten)
p = 0.001
(Edf=1; Ed=11.9%)
Macroinvertebrate richness
p = 0.012
(Edf=2.4; Ed=11.3%)
Organic mud (% pond coverage)
Effect on probability of
T. cristatus occurrence
p = 0.009
(Edf=1.7; Ed=10%)
Adjacent mixed woodland (% coverage)
p = 0.003
(Ed=9.8%)
Fish presence
p = 0.032
(Ed=7%)
Boulder and Clay/C2 (geology of pond area)
Effect on probability of
T. cristatus occurrence
p = 0.016
(Edf=1; Ed=6.1%)
Terrestrial habitat Diversity (Shannon index)
p = 0.012
(Edf=1; Ed=5.9%)
Slightly sloping bank (% coverage)
p = 0.021
(Ed=5%)
Sand and gravel/C2 (geology of pond area)
Effect on probability of
T. cristatus occurrence
p = 0.021
(Edf=1; Ed=4.9%)
Macrophytes (% coverage)
p = 0.019
(Ed=4.9%)
Humus-rich iron podsols (dominant soil around the pond)
p = 0.045
(Edf=1; Ed=3.6%)
Shore moss (% coverage)

51

Fig. 6 Estimated individual effect of each of the 12 selected predictor variables for the occurrence analyses, on the probability of occurrence of *T. cristatus*, determined from generalized additive models (GAM) made individually on each variable. Response curves are based on partial residuals and are standardized to have a mean probability of zero. The contour of the shaded areas is approximatly +/–1 SE relative to the main estimate, and hatch marks at the bottom are a descriptor of the frequency of data points along the gradient in continuous variables, or within each category for categorical variables. “Edf” means estimated degrees of freedom of the smooth curve. Edf=1 is equivalent to a linear relationship. Smoothing parameters were estimated by General Cross Validation error (Wood 2004). “Ed” means explained deviance. Variables are ordered from highest to lowest explained deviance.

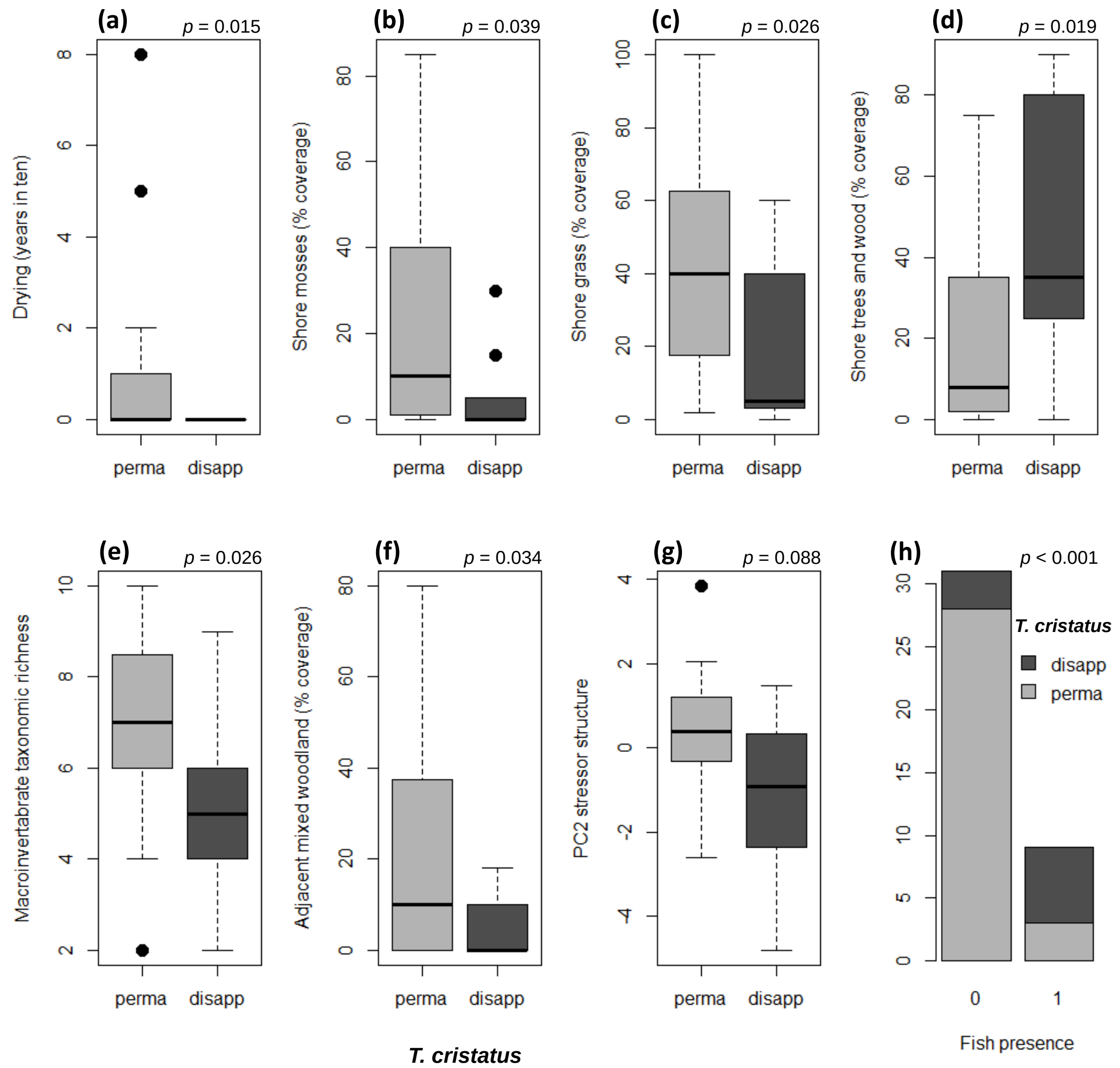


Fig. 7 Box plots and bar chart corresponding to the eight selected variables and factors for *T. cristatus* persistence analyses, showing (a) years in 10 when the pond dries up, (b) shore moss, (c) shore grass, (d) shore trees, (e) macroinvertebrate taxonomic richness, (f) adjacent mixed *Pinus sylvestris* - *Betula* woodland, (g) Principal Component 2 of the stressor structure, (h) fish presence, with permanence (perma, n = 31) and disappearance (disapp, n = 9) of *T. cristatus*. The line within each box marks the median, the bottom and top of each box indicate the 25th and 75th percentiles, the whiskers below and above each box indicate the 10th and 90th percentiles, and the points above and below the whiskers indicate the 5th and 95th percentiles. The *P* values of the U Mann-Whitney and χ^2 tests are given on the top of the boxes for numerical and categorical variables respectively. Note that PC2 for stressor structure is inversely correlated with the presence of adjacent roads (table 9).

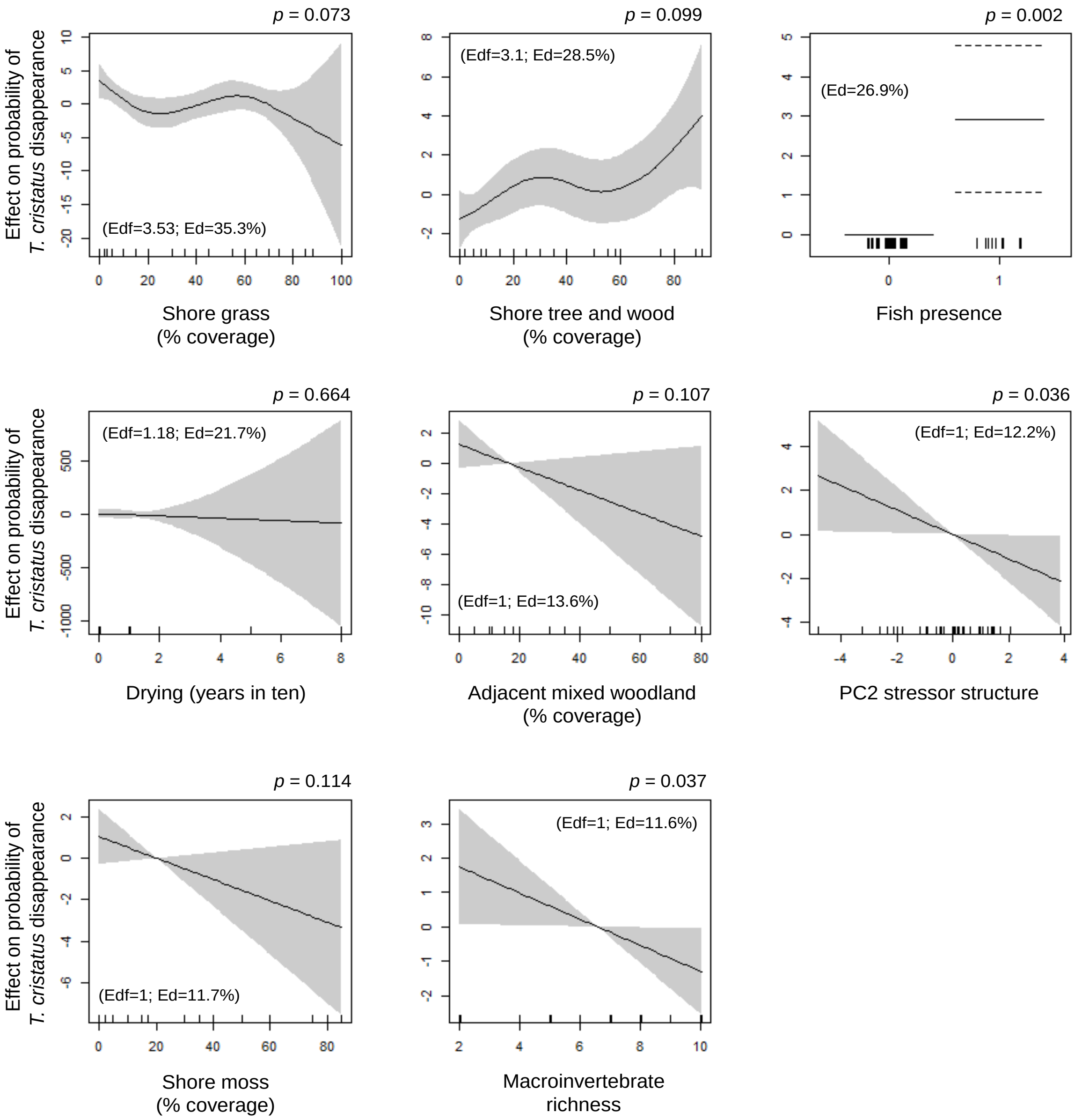


Fig. 8 Estimated individual effect of each of the eight selected predictor variables for the persistence analysis, on the probability of disappearance of *T. cristatus* determined from generalized additive models (GAM), made individually on each variable. Response curves are based on partial residuals and are standardized to have a mean probability of zero. The contour of the shaded areas is approximately +/–1 SE relative to the main estimate, and hatch marks at the bottom are a descriptor of the frequency of data points along the gradient in continuous variables, or within each category for categorical variables. “Edf” means estimated degrees of freedom of the smooth curve; Edf=1 is equivalent to a linear relationship. Smoothing parameters were estimated by General Cross Validation error (Wood 2004). “Ed” means explained deviance. Variables are ordered from highest to lowest explained deviance. Note that PC2 for stressor structure is inversely correlated with the presence of adjacent roads (table 9).

89

90