A study of the impact of fish on the distribution of amphibians in an agricultural region

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Abstract

Over the past decades, amphibians have been the focus of increasing concern in the scientific literature and popular press because of numerous reports of population declines, range constrictions, and extinction. Scientist are proposing many hypothesis but fish have been considered the most critical and widespread problem because they can be both competitors and predators of amphibians, especially on aquatic larvae. But the complex amphibians life cycle is influenced at several levels and it is important to take into account several other factors to illustrate fish impact. If not, results can be badly interpreted and biased by the influence of other important ecological variables. The present study was located in an agricultural region of Switzerland, where as far as we know, only one study was done in Switzerland about fish impact and including hydrological regime. The influence of fish was compared with the impact of other important variables, such as other amphibian larvae predators (Aeshidae larvae and *Dytiscus* beetles), pond size, vegetation cover, proximity of forests, of canals and of nearby breeding sites. This last variable was the most important one, with respect to the number of species influenced. This study couldn't highlight that fish have an important and generalised influence on amphibian distribution pattern in the study area. Almost every variable influenced at least one species. These results showed that in an equal landscape, species are distributed in function of different rules according to their ecology and needs for habitat selection. This highlight the importance and implications to take into account the multi scale-effect of landscape processes and habitat quality for conservation.

Introduction

Over the past decades, amphibians have been the focus of increasing concern in the scientific literature and popular press because of numerous reports of population declines, range constrictions, and extinctions (Semlitsch 2003, Collins and Storfer 2003, Blaustein et al. 2003, Kats and Ferrer 2003) and because they have the highest threat status of all terrestrial vertebrates, with significantly more species at risk than either birds or mammals (IUCN 2006, Stuart et al. 2004). Scientists propose several explanations for the decline of amphibians, such as habitat destruction and alteration (e.g. Blaustein et al. 1994, Dodd and Smith 2003, Alford and Richards 1999, Gardner et al. 2007), global climate change (Reading 2007), ultraviolet-B radiation (Blaustein et al. 2003), chemical contamination (Davidson et al. 2001, Sparling et al. 2001, Hayes et al. 2002), disease and pathogens (reviewed in Carey et al. 1999) for example

the fungus *Batrachochytrium dendrobatidis* (Lips et al. 2006, Pounds et al. 2006), commercial exploitation (Blaustein et al. 1994) and invasive species, such as fish. Fish have been considered the most critical and widespread problem because they can be both competitors and predators of amphibians, especially on aquatic larvae (Knapp and Mattews 2000, Pilliod and Peterson 2001, Van Buskirk 2003, Kats and Ferrer 2003, Anholt et al. 2005, Welsh et al. 2006, Hartel et al. 2007, Vredendurg 2004). It is now believed that fish can also act as vectors for disease (Dunham et al. 2004, Kiesecker et al. 2001, Mao et al. 1999).

All these explanations highlight the fact that amphibians are very sensitive animals to many ecological factors. Pond breeding amphibians have a complex life cycle with terrestrial adults and aquatic offspring. They undergo a major ontogenic niche shift whereby tadpoles and adults occupy two distinct habitats (Becker et al. 2007). Thus, they form an important link between terrestrial and aquatic habitat, with specific and strict ecological needs for each one (Resetarits 2005). In landscapes occupied by humans, as in this study, land use has resulted in a spatial separation between remnants of terrestrial habitat and breeding site (Dunning et al. 1992, Becker et al. 2007) and increasing migration constraints for adults and juveniles (Duellman and Trueb 1994, Mazerolle et al. 2005). Survival of aquatic stages is influenced by many factors such as pond hydroperiod, competition and predation, for example.

The determinants of habitat selection are particularly varied in species that use different environments during their lifetime, like amphibians (Pope and al. 2000, Resetarits 2005). The complex amphibian life cycle is influenced at three levels (Pope and al. 2000, Cushman 2006, Denoël and Lehmann 2006). The first level is the aquatic habitat, the place of breeding, egg laying and growth of larvae until metamorphosis (Duellman and Trueb 1994, Griffiths 1996). The second level is the landscape (Dunning et al. 1992). In many amphibians, this is a characterisation of the environment occupied after the reproduction period, i.e. terrestrial habitats used for displacements, foraging, hibernation and estivation (Laan and Verboom 1990, Guerrry and Hunter 2002, Hermann et al. 2005, Semlitsch 2007). The last level is the metapopulation structure (Hanski 1998) which is associated with the density of surrounding suitable breeding sites (e.g. Sjögren 1991, Vos and Stumpel 1995).

The aim of this study was to evaluate whether fish have an impact on amphibians in the Seeland. This influence of fish was compared with the influence of other factors that may operate at any of the three levels mentioned above. As far as we know, only one study has been made in Switzerland about fish impact and including hydrological regime (Van Buskirk 2003). For the present study, 56 artificial ponds built between 1995 and 2006 for a conservation project were sampled to establish amphibians occupancy according to fish

presence or absence in comparison with other important factors for amphibians distribution patterns.

Because of the complex life cycle of amphibians, it was important to take into account several other factors to illustrate fish impact. If not, results could be badly interpreted and biased by the influence of other important ecological variables. Nevertheless, several studies dealing with proving bad effect of fish on amphibians have only focused on this factor (e.g. Hartel and al. 2007, Eby et al. 2006, Brönmark and Edenhamn 1994, Anholt et al 2005, Bosch et al. 2006, Dunham et al. 2004, Denoel et al. 2005, Orizaola and Brana 2006, Vredenburg 2004, Hoffmann et al. 2004).

Other factors that may affect the distribution of amphibians were assessed at the levels of the pond, the landscape and the metapopulation. For the aquatic level, five variables were considered: firstly impact of presence or absence of predators such as fish, aeshnid dragonfly larvae and *Dytiscus* was measured. Secondly, characteristics of the ponds such as the vascular submerged vegetation cover and pond size in m2 were measured. For the landscape level, two variables were considered: forests and canals proximities. Finally, for the metapopulation level, measure of ponds connectivity with others was considered.

For every pond, presence/absence of amphibian species, amphibian survival, amphibian diversity and amphibian rarity were studied in function of the variables described above. Amphibian pond occupancy and amphibian survival were analysed with a binomial analysis for every variable, amphibian diversity and amphibian rarity were studied with a Poisson analysis. Detection probability was not taken into account for this study. To know whether data in this study were biased by nondetection, species detection probability was calculated with program PRESENCE (McKenzie et al. 2003) and then used to calculate how many surveys should have been required to demonstrate the presence of every species with a 95% probability. This minimum number of surveys was obtained with the probability model presented by McArdle in 1990.

Methods

Study area

The study area is located in Switzerland, in a region called Seeland located in the Cantons of Bern, Fribourg and Vaud, between the 3 lakes of Neuchâtel, Bienne and Morat. The precise extend of the study area forms a triangle. The base of this triangle runs north-east

from the village of La Thielle to the village of Münschemier at the north-west and its point is located at the south-east, near Neuhof (see Figure 1). It covers a total surface of 21,825 km^2.

The Seeland is a very flat region. The majority of the ponds are at 430 m elevation above sea level, the highest is at 440 m and there is no pond lower than 430 m elevation. The study area is located in an agricultural region. A big part of the fruit and vegetables of Switzerland are cultivated there (Lüscher, 2007). The entire Seeland used to be a marshy region in the past. People living there had very difficult life conditions: rivers overflowed very often because of the big quantity of alluvia deposits they were carrying along, making agriculture impossible, causing poverty and bringing illnesses (Nast 2006). In 1868, a first landscape modification was undertaken under the direction of de La Nicca (1794-1883, engineer). But the results of this modification were not conclusive so a second modification was made between 1962 and 1972 following the plans of Robert Müller (1908-1987, teacher at the Polytechnic School of Zurich ETH). This work made the region exploitable with several canals that are since then sources of water for the agriculture and furthermore, the course of the principal rivers is controlled. Those drastic landscape changes had a very strong negative impact on the environment. A lot of swamp-dwelling plant and animal species, including amphibians, suffered a big abundance diminution or some of them disappeared totally from the region. The Seeland will never be a pristine region again.

There are now several projects for nature conservation and ecological valorization in the Seeland region, including ponds and wetlands construction for protection and conservation of species depending on those habitats. Those projects are managed by "Siftung Biotopverbung Grosses Moos". This foundation was created in 1996 by "Freiburgischer Verband der Gemeinden des Seebezirks" and "Bernischer Regionalverband Erlach-östliches Seeland EOS" (new name: "seeland.biel/bienne"). Projects are carried out thanks to a collaboration based on partnership with farmers. "Siftung Biotopverbung Grosses Moos" is acknowledged in Switzerland as a model project and received the "Smaragdpreis" from the WWF in 2002 (Lüscher, 2007).
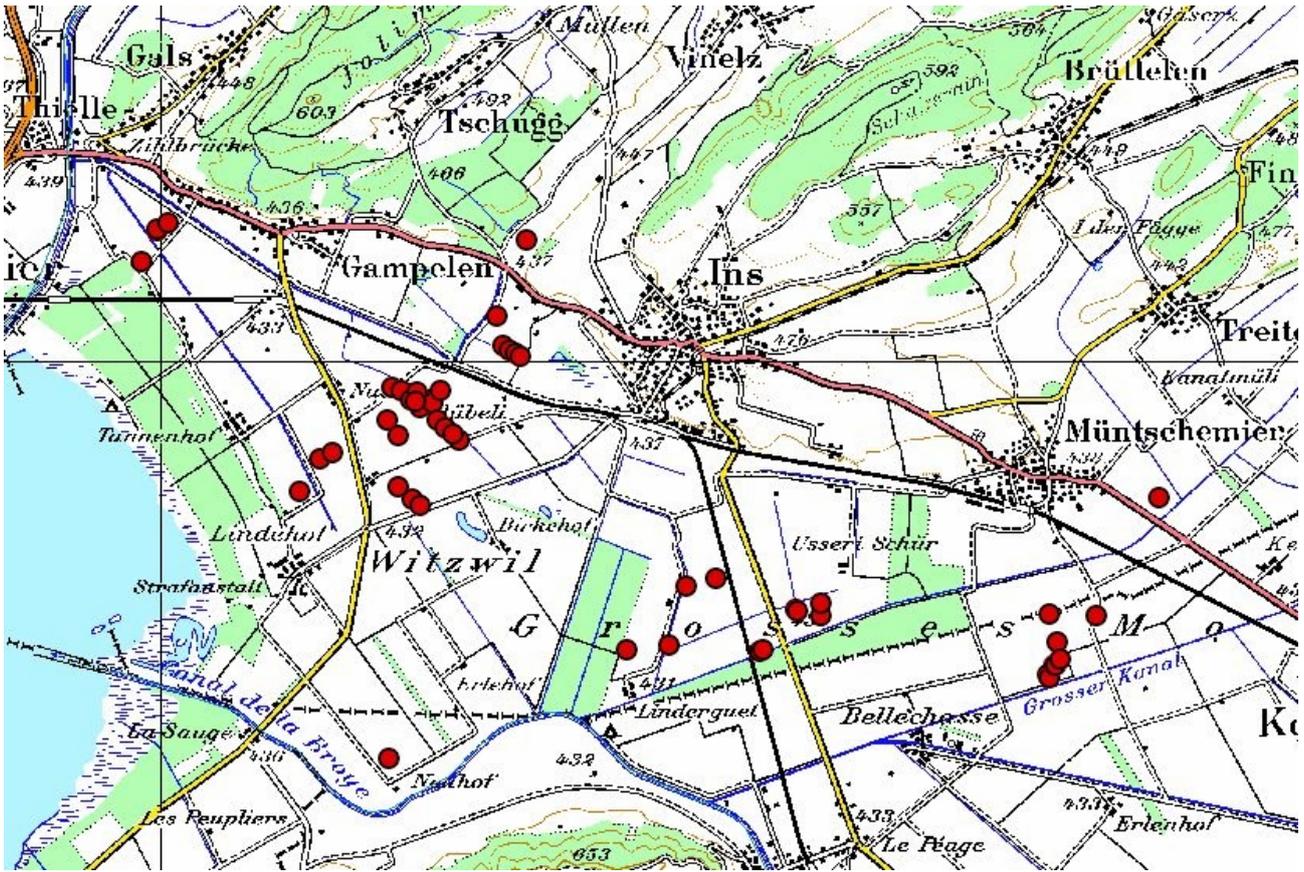
Figure 1 : Map of the study area, red dots are the 56 ponds included in the study

50 ponds were randomly chosen for this study and 16 were added during the first visit tour because located near some of the first random choice list and to get a bigger sample. 10 ponds were cancelled for the analysis because they were dry at every visit so they couldn't be taken into account for a herpetological study.

Data collection: Animals

Field work was done from 7th of March until 7th of July 2007. Data about animals were collected using 4 different methods: day observations for anuran adults, juveniles, calls and spawn, day capture with nets for anuran adults, juveniles and tadpoles and aquatic insects, night observations for anuran calls, Tomahawk fish traps for urodele adults and larvae, anuran adults, juveniles, tadpoles and subadults, aquatic insects and fish.

Tomahawk fish traps were put 3 times in almost every pond. They were put only twice in 3 ponds and only once in 1 pond because those 4 ponds were dry or not deep enough at one or two visits to put the traps 3 times. Traps were put in different microhabitats: in dense vegetation, in scattered vegetation, out of the vegetation, at the limit of the vegetation (at the edge or thrown from the edge and attached at the edge thanks to a long rope). The number of

traps was relative to the size of the ponds, not on the number of microhabitats. So, in big ponds, there could be more than 1 trap per microhabitat. Ponds where traps could never be put were not taken into account for the analysis.

Amphibian sampling was species specific to provide a presence/absence data set for every species. Abundance were not taken into account. Four development stages were differentiated for data collection: adults, calls and juveniles forming one category (which includes data from direct observations, trappings and call surveys), spawn (anurans only), larvae, subadult (anurans only). The category "subadult" for *Bufo bufo* was not studied because this stage for this species was found in only one pond, making comparison between ponds impossible. For urodeles, only adults and larvae were taken into account because of the difficulty to detect and determine eggs, laid one by one on the submerged vegetation, because juveniles use to stay in terrestrial habitats before reaching sexual maturity (Nöllert and Nöllert 2003) and because of the difficulty to differentiate larvae and subadults. *Hyla arborea* was only heard, no adults neither other stages could be observed during this study, so this species was studied thank to "calls" category only.

For the predators, every fish species was identified and noted. Aquatic insects taken into account into the database were those known and well documented in the literature as natural predators of spawn, tadpoles and subadults: aeshnid dragonfly larvae and *Dytiscus* beetle adults and larvae forming one category (Semlitsch 1993, Anholt et al. 2005, Werner and McPeek 1994, Imboden 1976, Mulhauser and Monnier 1995, Van Buskirk 2005).

Other predators, such as other Odonata larvae (Libellulid larvae found in 38 ponds, and Agriid larvae found in 25 ponds), Notonectidae (found in 49 ponds), snakes (*Natrix natrix* found in 5 ponds) and crayfish (*Orconectes limosus,* found in 3 ponds) were not taken into account for the analysis to limit number of variables according to the sample size .

This database for amphibians, aquatic insects and fish was used to make a present/absence statement for every pond.

Data collection: ponds characteristics

Pond size plays several roles such as influencing water temperature and hydroperiod (Warren and Büttner 2007). This variable was estimated walking around the ponds and counting steps, considering 1 step as 1 meter. Then, using general geometric form of each pond or of each part of an irregular pond, the area could be calculated. This was done at the

end of June, after mowing period to be able to walk around the ponds without being biased by the difficulty of walking in high vegetation.

Submerged vegetation cover reflects an immerged network of stems creating structurally complex habitats which offer shelter for spawn, larvae and subadults (Denoël and Lehmann 2006, Mulhauser and Monnier 1995). Vegetation also represents a source of food (Imboden 1976). This variable was considered according to a cover scale : no vegetation (0), a bit (1), medium (2), a lot (3). Some parts of the surface covered with vegetation = a bit; half of the surface covered with vegetation or more than the half but uncovered surfaces still visible from the edge = medium; the surface was not visible anymore from the edge = a lot. Plants taken into account to evaluate vegetation cover were emergent plant species: *Phragmites australis* and *Typha latifolia.*

Data collection: landscape variables

Forest proximity is important to provide terrestrial habitat to amphibians and for migration constraints (Pope et al. 2000, Becker et al. 2007, Semlitsch 2007, Mazerolle et al. 2005, Denoël and Lehman 2006). Canals can be a pond colonization sink for fish (Ray et al. 2004). Pond connectivity can augment pond colonisation probability and maintain a viable metapopulation (Pellet et al. 2007, Compton et al. 2007) or community (Richter-Boix et al. 2007). Those variables were calculated with a GIS system. Land use data were derived from the VECTOR25, the vector format of the 1:25'000 topographical map of Switzerland provided by the Swiss Federal Office of Topography (Swisstopo). Precision of maps is approximately 3-8 meters. For proximity of canals a circle of 100 m diameter was drawn around every pond and the length of the canals into this circle was then calculated. For proximity of forest, a circle of 1 km diameter was drawn around every pond and the total surface covered by forests into this circle was then calculated.

Data collection: metapopulation variable

For ponds connectivity, distances to nearby ponds ($d_{1,2,...,n}$) were measured for every selected pond in a total area including 5 kilometres around the study area, to take into account every amphibian population that might occupy it. The connectivity index for every pond was then calculated as follows:

$$\text{Connectivity} = \Sigma \exp(-d_i)$$

Materials

Amphibians were captured with Tomahawk fish traps and nets usually used to clean pools. A plastic bottle was always put into the traps to avoid the traps sinking. It is important for the animals in the traps to be able to reach the surface to get oxygen. The nets are triangular with 2 hard and 1 soft edges, the length of the handle is adjustable. To keep the individuals long enough for the determination they were put in a bucket with water, big enough to avoid frogs jumping out.

Fish and aquatic insects were captured with Tomahawk fish traps and also put into the bucket with water for the determination. Fish traps are very fragile so they often used repairing. The holes were sewed with a linen in nylon and a cloth usually used by the farmers to protect their cultivations from bad weather and insect pest.

Data analysis

The analysis was made in 2 steps for every amphibian species separately. Firstly, all stages were pooled to create a data set where the species in general were either present or absent in the ponds. Ponds amphibian diversity and rarity were also analysed. Amphibian diversity is the total number of amphibian species in every pond, amphibian rarity was calculated as follow. Presence (1) or absence (0) of every species was first divided by the number of specific sampling occasions. Those results were added up for every pond and then multiplied by the total number of ponds (56).

The second step was to study amphibian survival. For anurans, ponds where spawn had been detected were selected. Presence/absence of subadults in those ponds was then analysed. Ponds where spawn had not been detected but subadults had been observed were also taken into account for survival analysis. Indeed, if an advanced development stage is present, reproduction did occur in those ponds but previous development stage were not detected. So those ponds had also to be considered for the analysis. For *Bufo bufo*, presence/absence of larvae was analysed because subadults had been found in only one pond. For urodeles, ponds where adults had been caught were selected. Presence/absence of larvae in those ponds was then analysed. Ponds where adults had not been caught but larvae had been found were also taken into account for survival analysis for the same reason as for anurans.

Amphibian species presence/absence, amphibian diversity, amphibian rarity and amphibian species survival were analysed according to several sites covariates: presence/absence of fish, of aeshnid dragonfly larvae, of *Dytiscus* adults and larvae, pond size, vegetation cover, proximity of canals and of forest, connectivity of every pond with the others. Table 1 lists those variables.

Table 1 : list of variables taken into account for the analysis

Variables	Specifier
SITE COVARIATES	
Fish	Absent, present (0,1)
Aeshnid dragonfly larvae	Absent, present (0,1)
Dytiscus	Absent, present (0,1)
Ponds size	m^2
Vegetation cover	nothing, a bit, medium, a lot (0,1,2,3)
Canals proximity	m
Forest proximity	m^2
Connectivity	-

Presence/absence of amphibian species in every pond and presence/absence of subadults or larvae in ponds where previous development stages had been found was studied with a binomial analysis (logistic regression) for every variable. P-value for every variable was significant (*) if equal or lower than 0.05, very significant (**) if equal or lower than 0.01 and extremely significant (***) if equal or lower than 0.001. P-values between 0.05 and 0.1 are marginally non significant but represent a tendency. No Bonferroni correction was applied to account for multiple testing (the same predictor variables are tested with multiple species).

Amphibian diversity and amphibian rarity were studied with a Poisson analysis and P-value significances for every variable were considered as with the binomial analysis.

It was impossible to estimate true site occupancy rates, since a species may be undetected either because it was truly absent or simply overlooked. The problem of "false absence" can be reduced if sites are sampled repeatedly (e.g., McKenzie et al. 2002). In this study, every of the 56 ponds were visited between 8 or 11 times (depending on how many times Tomahawk fish traps could be placed). To know how many visits (N) should have been done to be sure with 95% probability to avoid "false absence" for every species, species detection probability (p) was estimated with the program PRESENCE (McKenzie et al. 2003). The species specific detection probabilities were then used to estimate necessary number of visits to avoid false

absence with the probability model presented by McArdle in 1990:

$$N = \frac{\log(0,05)}{\log(1-p)}$$

This results was then compared with the number of visits carried out in the present study. This number was specific to every amphibian species. Indeed, every species has different seasonality and data collection method was not the same for anurans and for urodeles. This number was obtained considering only the portion of visits between the dates of first and last detection exclusive. Truncating data in this manner ensures that species were available to be detected throughout that portion of monitoring period (McKenzie et al. 2002).

Results

Animal observations

7 amphibian species were found in the study area: the common frog *Rana temporaria*, the common toad *Bufo bufo*, the green frog *R. esculenta* complex (*R. esculenta, R. lessonae* and *R. ridibunda*) the alpine newt *Triturus alpestris*, the smooth newt *T. vulgaris*, the palmate newt *T. helveticus* and the tree frog *Hyla arborea*.

R. temporaria was detected in 25 % of the ponds (14/56), 8 of which containing fish and 6 of which without fish (see figure 2). Detection of the different life stages was as follows: adults in 7,14 % (4/56), spawn in 14,29 % (8/56), larvae in 12,5% (7/56) and subadults in 8,93 % (5/56).

Bufo bufo was detected in 25 % of the ponds (14/56), 11 of which containing fish and 3 of which without fish (see figure 3). Detection of the different life stages was as follows: adults in 17,86 % (10/56), spawn in 5,36 % (3/56), larvae in 14,29 % (8/56).

R. esculenta complex was detected in all the ponds. Detection of the different life stages was as follows: adults were detected in all the ponds, spawn in 60,71 % (34/56), larvae in 82,14% (46/56) and subadults in 39,29 % (22/56). *T. alpestris* was caught in 26,79 % of the ponds (15/56), 4 of which containing fish and 11 of which without fish (see figure 4). Detection of the different life stages was as follows: adults in 23,21 % (13/56) and larvae in 12,5 % (7/56).
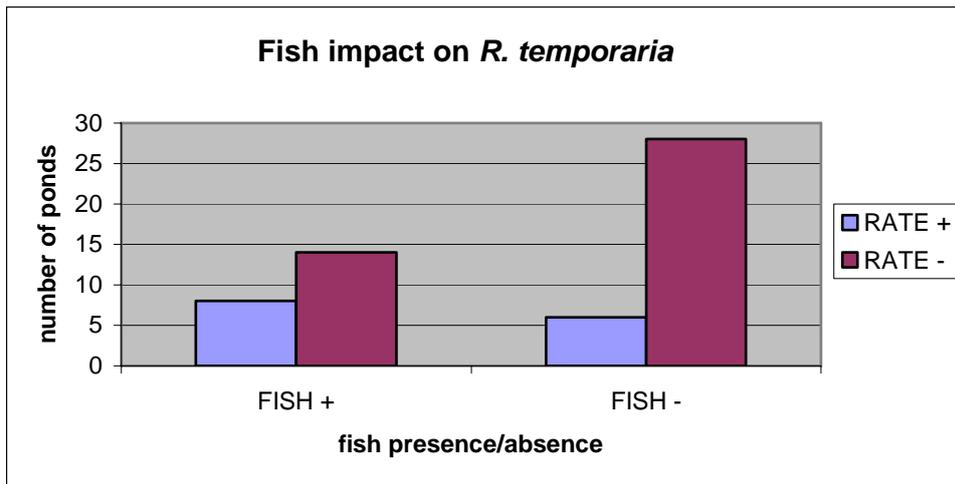
Figure 2: Distribution of *R. temporaria* (RATE) in ponds with (FISH +) and without fish (FISH -) (n=56). RATE + means that common frogs were present, RATE - means that they were absent from the ponds. Blue bars are ponds that had reproducing common frog during the present study; violet bars are ponds with no recording of common frog presence.
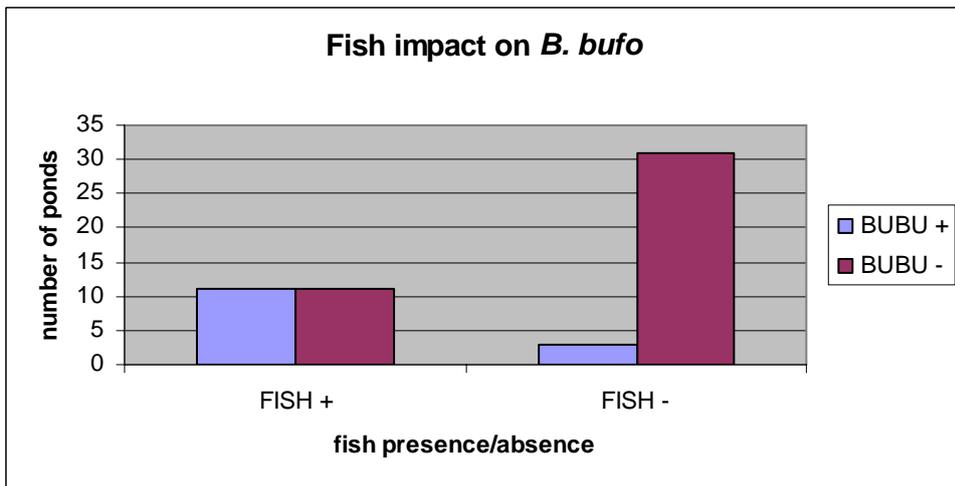
Figure 3: Distribution of *B. bufo* (BUBU + or -) in ponds with (+) and without (-) fish (n=56). Significance of bar colours is the same as in figure 2.
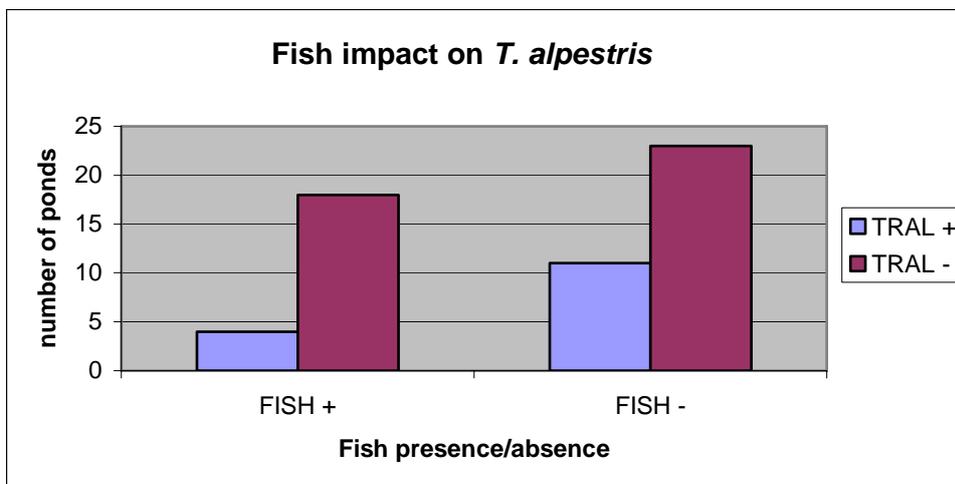
Figure 4: Distribution of *T. alpestris* (TRAL + or -) in ponds with (+) and without (-) fish (n=56). Significance of bar colours is the same as in figure 2.

T. vulgaris was caught in 19,64 % of the ponds (11/56), one of which containing fish and 10 of which without fish (see figure 5). Detection of the different life stages was as follows: adults in 17,86 % (10/56) and larvae in 14,29 % (8/56).
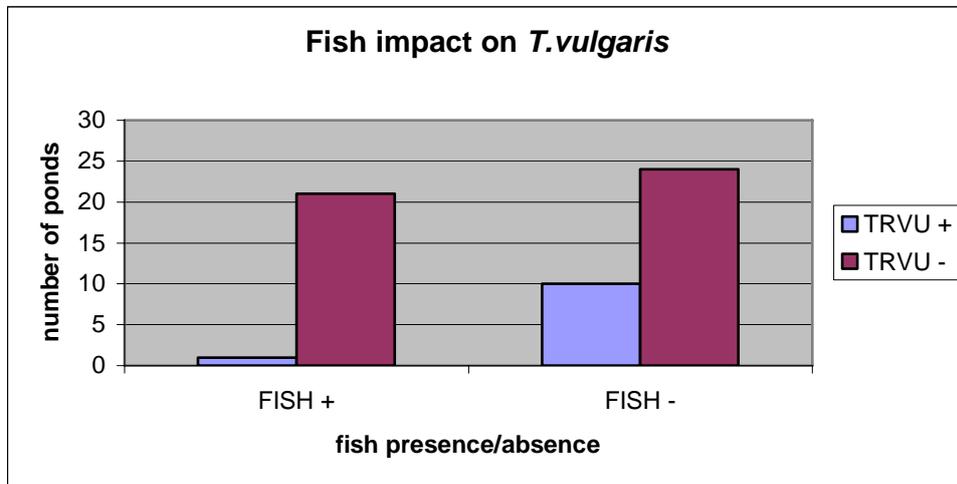
Figure 5: Distribution of *T. vulgaris* (TRVU + or -) in ponds with (+) and without fish (-) (n=56). Significance of bar colours is the same as in figure 2.

T. helveticus was caught in 7,14 % of the ponds (4/56), all without fish (see figure 6). Detection of the different life stages was as follows: adults and larvae in 3,57 % (2/56). Finally, *H. arborea* was heard in 7,14 % of the ponds (4/56), all without fish (see figure 7).
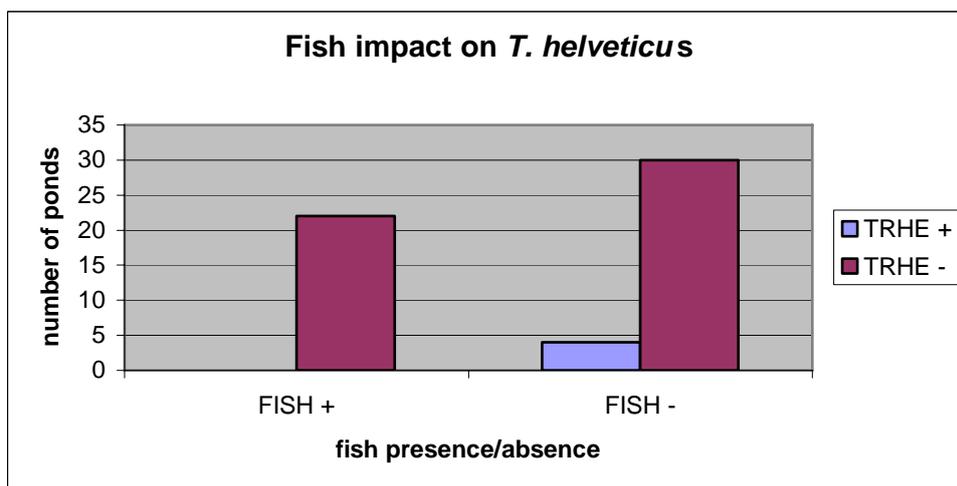
Figure 6: Distribution of *T. helveticus* (TRHE + or -) in ponds with (+) or without (-) fish (n=56). Significance of bar colours is the same as in figure 2.
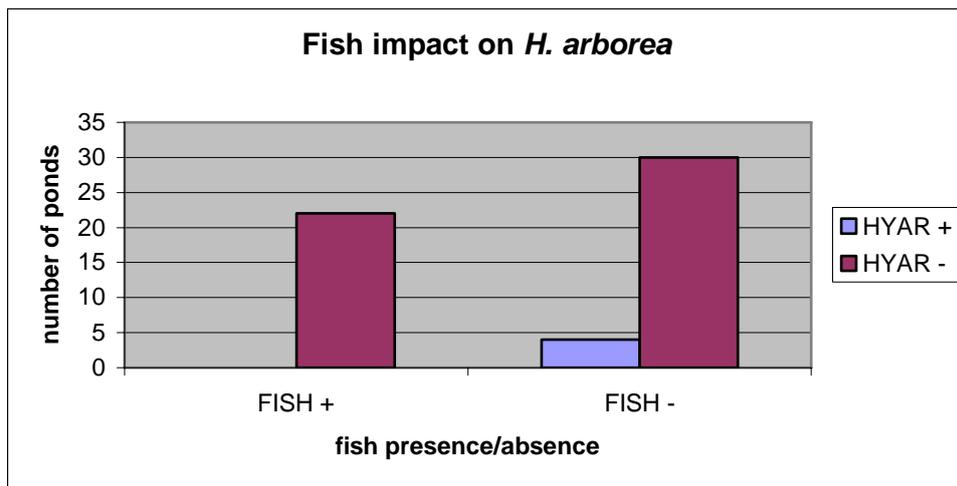
Figure 7: Distribution of *H. arborea* (HYAR + or -) in ponds with (+) or without (-) fish (n=56). Significance of bar colours is the same as in figure 2.

13 fish species were found during this study: *Rutilus rutilus, Scardinius erythrophtalmus, Cobitis taenia, Tinca tinca, Leuciscus cephalus, Perca fluviatilis, Gasterosteus aculeatus, Rhodeus sericeus amarus, Esox lucius, Carassius carassius, Cyprinus carpio, Alburnus alburnus* and *Gobio gobio.* Fish were caught in 39,29 % of the ponds (22/56).

Aeshnidae larvae were found in 73,21 % (41/56), *Dytiscus* sp. were found in 62,5 % (35/56).

Amphibian ponds occupancy

Variables significant for *R. temporaria* occupancy are connectivity (P-value = 0.00982 **) with a negative effect (estimate = -3.630e-01, std. error = 1.406e-01) and there is a tendency for a positive impact of increasing pond size (P-value = 0.07271, estimate = 7.624e-04, std. error = 4.248e-04).

Variables significant for *B.bufo* occupancy are fish (P-value = 0.00856 **) with a positive effect (estimate = 3.257, std. error = 1.239) and connectivity (P-value = 0.02768 *) with a negative effect (estimate = -3.573e-01, std. error = 1.623e-01). There is a tendency for a negative impact of *Dytiscus* (P-value = 0.07990, estimate = -2.098, std. error = 1.198).

There isn't any significant variable to explain *R. esculenta* complex occupancy because this species occurred in all the ponds.

Only canals proximity explain significantly *T. alpestris* occupancy with a negative effect (P-value = 0.0430 *, estimate = -9.052e-03, std. error = 4.474e-03).

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Forest proximity explains significantly *T. vulgaris* occupancy with a positive effect (P-value = 0.0340 *, estimate = 6.457e-06, std. error = 3.045e-06). There are tendencies for a negative impact of fish (P-value = 0.0945, estimate = -3.832, std. error = 2.292) and for a positive impact of increasing vegetation cover (P-value = 0.0607, estimate = 1.609, std. error = 8.582e-01).

There isn't any significant value to explain *T. helveticus* and *H. arborea* occupancies.

Connectivity has a negative impact on diversity (P-value = 0.03079 *, estimate = -5.929e-02, std. error = 2.745e-02) and on rarity (P-value = 0.0160 *, estimate = -1.011e-01, std. error = 4.198e-02). There is a tendency for a negative impact of canals proximity on rarity (P-value = 0.0722, estimate = -3.172e-03, std. error = 1.764e-03).

Results for significant variables explaining amphibian ponds occupancy are visible in figures 8 to 12. Values obtained with the logistic regression were transformed from logit to probabilities.
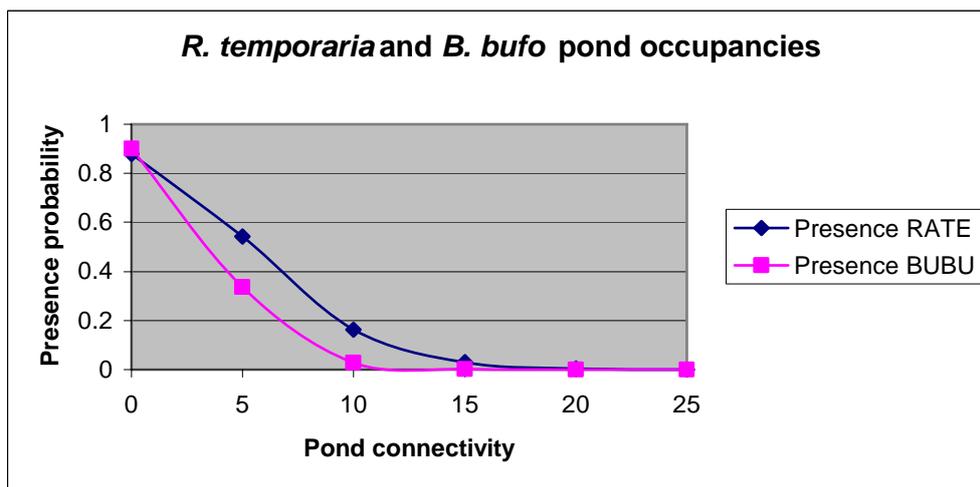
Figure 8: pond occupancy probabilities for *R. temporaria* (RATE) and *B. bufo* (BUBU) explained by pond connectivity
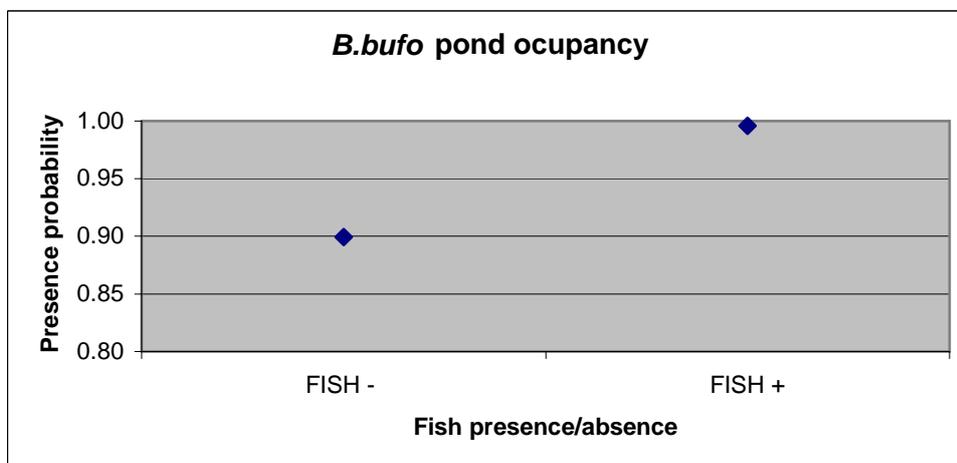
Figure 9: Pond occupancy probability for *B. bufo,* explained by fish presence (FISH +) or absence (FISH –). Pond occupancy probability in fishless ponds was 0.899 and 0.996 in fish occupied ponds.
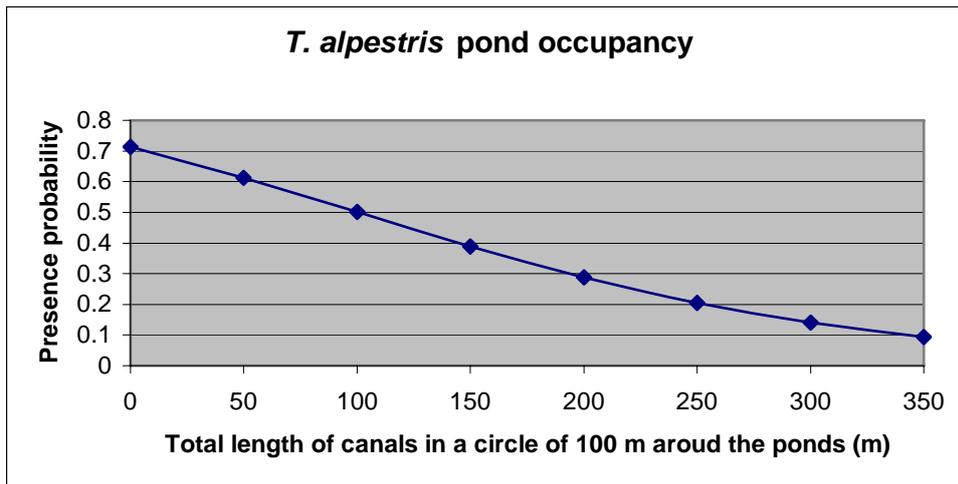
Figure 10: pond occupancy probability for *T. alpestris* explained by canal proximity
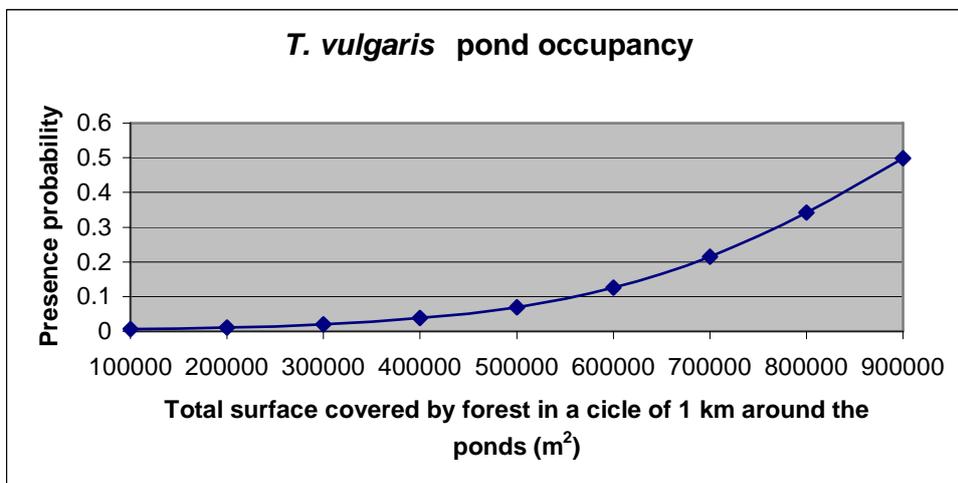
Figure 11: pond occupancy probability for *T. vulgaris* explained by forest proximity
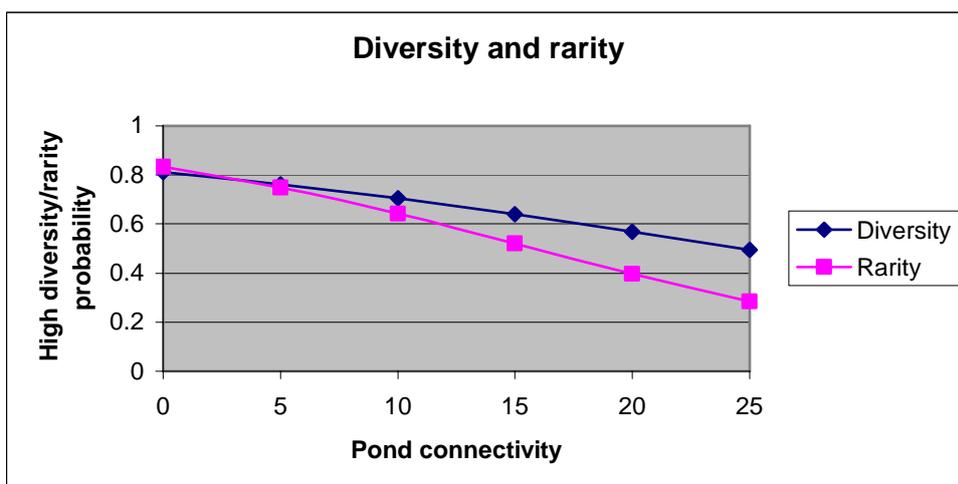
Figure 12: probability of high diversity and rarity in ponds explained by pond connectivity

Survival

There isn't any significant variable to explain survival of *R. temporaria, B. bufo, T. alpestris, T. vulgaris* and *T. helveticus*. *R. esculenta* complex survival was explained significantly by pond size with a positive effect (P-value = 0.0478 *, estimate = 1.278e-03, std. error = 6.455e-04) and by connectivity with a negative effect (P-value = 0.0193 *, estimate = -3.653e-01, std. error = 1.562e-01).

Results for significant variables explaining *R. esculenta* complex survival are visible in figures 13 and 14. Values issued from the analysis with R were transformed from logit to probabilities.
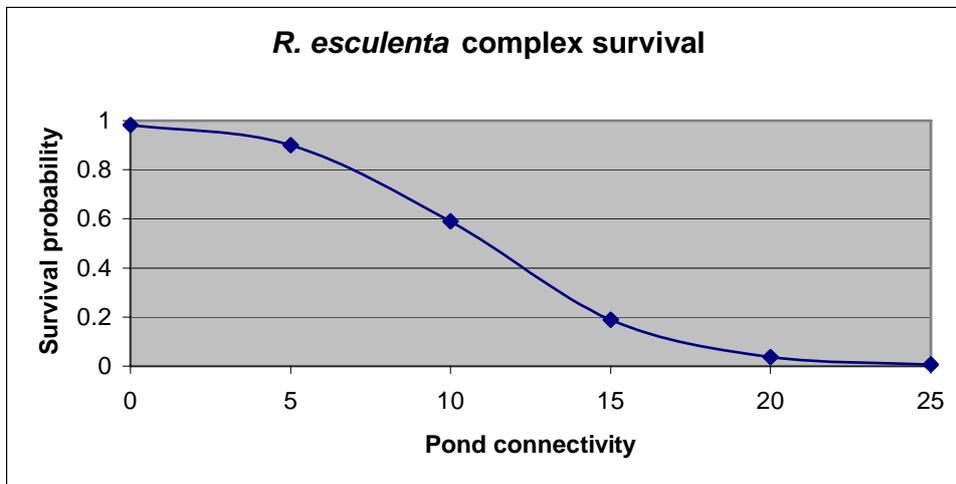
Figure 13: survival probability for *R. esculenta* complex immature stages explained by pond connectivity
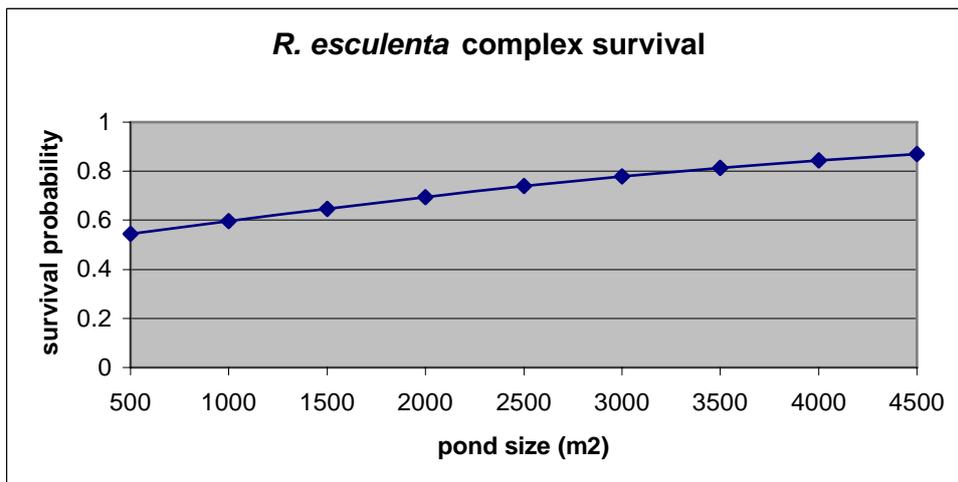
Figure 14: survival probability for *R. esculenta* complex immature stages explained by pond size

Number of visits

Rana temporaria was observed between the 7th of March (spawn) to the 6th of June (subadult). Day observations, captures with tomahawk fish traps and pool net and night listenings for this species were carried out 6 to 10 times in every pond during this period, with an average of 8,25 visits.

Bufo bufo was observed between the 10th of March (adult and spawn) to the 3rd of May (larvae). Day observations, captures with tomahawk fish traps and pool net and night listenings for this species were carried out 4 to 7 times in every pond during this period, with an average of 5,3215 visits.

R. esculenta complex was observed between the 13th of March (adult) to the 7th of July (adult, larvae, subadult). Day observations, captures with tomahawk fish traps and pool net and night listenings for this species were carried out 8 to 10 times in every pond during this period, with an average of 9,0357 visits.

Triturus alpestris and *T. vulgaris* were observed between the 11th of April (adults) and the 6th of July (larvae). Captures with tomahawk fish traps for both species were carried out 1 to 3 times in every pond during this period, with an average of 2,875 trap layings.

T. helveticus was observed between the 28th of April (adult) and the 6th of July (larvae). Captures with tomahawk fish traps for this species were carried out 0 to 3 times in every pond (pond 22 did not contain enough water after the 20th of April so tomahawk fish traps could only be put once on the 20th of April) during this period, with an average of 2,0357 trap layings.

Hyla arborea was heard between the 3rd of May and the 20th of June. Night listenings for this species were carried out 1 to 3 times for every pond during this period, with an average of 1,3393 visits.

Table 2 compares those results with the necessary number of visit that should have been made to be sure with a 95% confidence that a species is absent from a pond. Results implies that for many species (apart from *R. esculenta* complex), presence was overlooked.

Table 2 : Necessary number of visits (N) to be sure with a 95% confidence that a species is absent from a pond

Species	average number of visits in the present study	detection probability	N
Rana temporaria	**8,25**	0,3039	**8,2663**
Bufo bufo	**5,3214**	0,3268	**7,5689**
R. esculenta complex	**9,0357**	0.6646	**2.7416**

Table 2 : continuation

Species	average number of visits in the present study	detection probability	N
Triturus alpestris	**2,875**	0.3545	**6.8428**
T. vulgaris	**2,875**	0.6280	**3.0287**
T. helveticus	**2,0357**	0.2411	**10.857**
Hyla arborea	**1,3393**	0.7723	**2.0244**

Discussion

Amphibian pond occupancy

The results show that fish don't seem to be the most important factor to explain amphibian pond occupancy in the Seeland. Fish have a significant positive effect on pond occupancy for *Bufo bufo* and tend to negatively affect pond occupancy in *Triturus vulgaris*. For the other predation variables, aeshnid dragonfly larvae don't seem to influence pond occupancy and *Dytiscus* tend to influence negatively *B. bufo* occupancy. The most important factor, with respect to the number of species influenced, is a metapopulation variable: pond connectivity being an explaining variable with a negative effect in five cases: for two species (*Rana temporaria* and *Bufo bufo*), for diversity, for rarity and for *R. esculenta* complex survival. The other landscape variables influence two species (canals proximity influence negatively *T. alpestris* occupancy and forests proximity influence positively *T. vulgaris* occupancy) and canals proximity tend to influence rarity negatively. Characteristics of the ponds tend to influence two species occupancy (pond surface with a tendency to influence positively *R. temporaria* occupancy and vegetation cover with a positive tendency to influence *T. vulgaris* occupancy) and have a positive effect on *R. esculenta* complex survival (pond surface).

The reason of the lack of significant variables to explain *T. helveticus* and *Hyla arborea* occupancies is that those species seem to be very rare in the study area. Percentage of pond occupancy is too low in both cases, this means that significant effects of explanatory variables are unlikely. The small number of immature stages observations also explain the lack of significant variables to analyse amphibians survival, except for *R. esculenta* complex.

There isn't any significant variable to explain *R. esculenta* complex pond occupancy because this species was always present. So none of the variable had a positive or a negative effect on this species.

Aquatic level variables: predators

Positive effects of fish on bufonid toads are well documented (Van Buskirk 2003, Orizaola and Brana 2006, Welsh et al. 2006, Hartel et al. 2007). Indeed, the unpalatibility of toad tadpoles due to their toxicity is a good defence against fish predation (Gunzburger and Travis 2005, Manteifel and Reshetnikov 2002, Crossland and Alford 1998). Toads are often found in ponds occupied by fish, probably to avoid competition with other amphibians species. Fish can also have a indirect positive effect on some amphibians due to differential predation on their competitors and predators, like dragonfly larvae (Werner and MacPeek 1994, Smith et al. 1999, Maezono and Miyashita 2003).

This study could only demonstrate a negative effect of fish on urodeles with the case of *Triturus vulgaris*. This can be explained by predation. Small fish like *Perca fluviatilis* for example cannot prey on adults amphibians because of gape limitation but frequently eat their eggs and larvae or cause severe injuries in amphibian larvae (Hartel et al. 2007). The large sized fish like *Esox lucius* can consume even the postmetamorphic stages of amphibians (Hartel et al. 2007). Beside direct predation, fish may negatively affect the abundance of aquatic invertebrates (such as crustaceans, oligochaetes and chironomids Reshetnikov 2003) that may constitute a major part of the prey of newts (Joly and Giacoma 1992).

It could be interesting to differentiate fish in two categories: predatory and non-predatory ones to see if it makes a difference in the results. If not, it would mean that the different kinds of fish present in the Seeland feed on amphibians larvae, even if considered as non-predators. Such results about the same fish species as found in the present study were already published by Breuer (1992), Meier (1995), Gebhart (1983), Glandt (1985), Bushholz (1989), and Hohnej (1999) (authors cited by Laufer et al. (2007)).

Canal proximity is a landscape variable but was expected to indirectly influence negatively amphibians occupancy because being an explanation for fish presence (Ray et al. 2004), that's why it is discussed in this section. Canal proximity did influence negatively *Triturus alpestris* occupancy and rarity. The genus *Triturus* is also known to be an amphibian larvae predator (Van Buskirk 2001). If canal proximity really illustrates fish presence in Seeland ponds, fish could have two strong negative impacts on *T. alpestris* : predation and competition. The same condition can explain the effect of this variable on rarity. Indeed, the rarest species in the Seeland, *Hyla arborea* and *T. helveticus* were never detected in fish occupied ponds. Of course, in those cases, species were too rarely found to prove that fish

especially affect those species but other studies about *H. arborea* obtained similar results: this species only being found in fishless ponds (Van Buskirk 2005, Resetarits 2005, Orizaola and Braña 2006). Other studies show a drastic fall of *H. arborea* occurrence score in ponds occupied by fish in comparison with fishless ones (Van Buskirk 2003, Brönmark and Edenhamn 1994). The study of Orizaola and Brana in 2006 showed an important difference in *T. helveticus* occurrence between lakes with or without Salmonids, more than 80% of fishless ones containing *T. helveticus* against 35% of fish occupied ones. Other urodeles were absent in lakes with fish. Denoël and Lehmann(2006) also obtained similar results: newts always found in fishless ponds. Those results confirm negative effect of fish on both the rarest species and on urodeles in general, which can explain negative effect of canal proximity found in the present results, even if data are missing.

Nevertheless, there were no effect of canals on fish pond occupancy (logistic binomial regression with R showed that this variable was not significant: P-value = 02248). Actually, pond size was the only variable explaining significantly fish pond occupancy (P-value = 0.0162 *, estimate = 2.292e-03, standard error = 9.528e-04).

Invertebrate predators had an impact only on one species: *Dytiscus* sp. tending to influence negatively occupancy of *B. bufo*. This can probably be explained by predation. Field observations in the present study revealed that predator aquatic insects are not affected by unpalatibility of bufonid larvae (an aeshnid dragonfly larvae was seen eating a tadpole of *B. bufo*).

Aquatic level variables: ponds characteristics

The importance of pond size on amphibians presence has been well documented (e.g. Dickam 1987, Laan and Verboom 1990, Bradford et al. 2003). In this study, the effect of this variable could be demonstrated for two species, both being positively correlated with increasing pond size: *R. temporaria* and *R. esculenta* complex survival. The main mechanisms through which pond size affect amphibians might be the effects of size on hydroperiod and water temperature. Indeed, these variables don't affect every species in the same manner, amphibians frequenting both small and large ponds depending on their biology (Mazerolle et al. 2005, Van Buskirk 2005). For example, species with long larval periods such as waterfrogs are infrequent in ephemeral ponds (Griffiths 1996, Abt Tietje and Reyer 2004) whereas the species with the shortest larval period (*Bombina variegata*) avoid permanent ponds, because permanent ponds don't get warm quickly enough for the larval development, contrary to

ephemeral smaller ponds (Barandun and Reyer 1997). Increasing pond size can also affect amphibians negatively, because containing more predators (Pearman 1993 and 1995). Pond size influence amphibians distribution patterns in several ways. This might explain why results didn't highlight an important influence of this variable to understand amphibian pond occupancy in the Seeland. Small ponds may dry and big ponds may contain high predators density.

Vegetation plays several roles in aquatic systems. It constitutes the base of the food chain, it produces oxygen which is important for the gobal aquatic system equilibrium, it is a support for amphibians spawn and it offers shelters to many prey species like amphibian larvae (Hecnar and McCloskey 1997, Babbitt and Tanner 1997, Denoël and Lehmann 2006). Those reasons explain the positive tendency of observed vegetation cover on *T. vulgaris*. It confirms results of Hartel et al. (2007) where the occurrence of six amphibian species was positively associated with emergent vegetation. They obtain the same results for amphibian species richness. Newts seem to be even more associated with shady ponds than anurans (Van Buskirk 2005, Denoël and Lehmann 2006)

Landscape level variables

Percentage forest cover within a buffer with a 1 km radius was important for *T. vulgaris*. It is important for animals with a complex life cycle, with different habitats during the year, like amphibians, to meet all their resource needs in their environment (Pope et al. 2000). Pond-breeding amphibian species differ in their preference for terrestrial habitats. For many of them, forests are an important resource for hibernation, foraging and migrations (Guerry and Hunter 2002, Houlahan and Findlay 2003, Porej et al. 2004, Hermann et al 2005). Access to resource patches must be appropriate at the individual level (Pope et al. 2000). It is not surprising to find the importance of forest proximity for a newt species. Indeed, the three *Triturus* species found in this study usually have smaller migration distances than anurans (Nöllert and Nöllert 2003). Marnell (1998) showed the importance of forests areas near breeding sites for *T. vulgaris*, particularly through the availability of terrestrial refugia (e.g. logs). Denoël and Lehmann (2006) showed the importance of forest in sustaining large newt populations. Terrestrial buffer zone around breeding sites are thus essential to allow amphibians to complete their life cycle (Semlitsch 1985, Schabetsberger et al. 2004).

Metapopulation level variable

A good explanation for the widespread negative effect of connectivity can be a geographical component of the Seeland. Many constructed ponds are either single highly isolated or form clusters of ponds where distance between them is very small. Each pond in such clusters were considered as independent units. In an area where ponds are either very connected or very isolated, it can be presumed that amphibians don't really have the choice between ponds with or without fish or with or without other variables. Indeed, very connected ponds might share several identical characteristics. In a very flat region like the Seeland, it is particularly the case: when it is raining, a lot of ponds are not separated anymore, forming together bigger unique ponds. In the 56 selected ponds of this study, there are 14 groups of ponds that are connecting their surface after raining periods. There is then a way for aquatic animals such as predators and amphibian larvae to pass from one pond to another. Those ponds, because located near from one another, also share same landscape characteristics. Those 14 groups of ponds gather 36 of the 56 ponds (64,28 %). There were 3 totally isolated ponds (5,36 %) and 8 groups of nearby ponds that were separated by a physical barrier (such as mound or small hills). Those 8 groups of ponds are not connected with their water but share at least same landscape characteristics. Those 8 groups gather 17 ponds (30,36 %).

The first arrived species (*R. temporaria*), breeding earliest, is going to occupy the first encountered ponds in its spring migration from overwintering sites to breeding sites. Laurila and Aho (1997) showed that this species doesn't choose breeding sites to avoid predation on tadpoles. If breeding sites are located near other available ones, species breeding later in the season might occupy the nearby ponds to avoid competition (Warren and Büttner 2007). This would result in a positive effect of connectivity on diversity. But in isolated ponds, species don't have the opportunity to breed in nearby ponds. It can be presumed that amphibians will breed in those ponds, even if they are already occupied by competitors and/or not presenting optimal breeding conditions (for example presence of predators, small pond with a risk to dry), and thus to avoid a risky extension of migration to an other breeding site. First of all, quality of other breeding sites is not guaranteed, secondly, distance between breeding sites can represent a lot of risks, for example water loss, heat stress, predators on the way, roads (O'Connor and Tracy 1992, Tracy et al. 1992, Rothermel and Semlitsch 2002). Pond isolation in pond-breeding amphibians has also been suggested as a cause of decline (Dodd and Smith 2003). Little is known about amphibians migration processes. Most amphibians species probably lack true navigation ability (Sinsch 1990). Perception of olfactory cues to detect

ponds likely occurs only at relatively short distances (McGregor and Teska 1989, Joly and Miaud 1993). Thus it can be presumed that philopatry has probably evolved among amphibians because of high cost of dispersal (Semlitsch 2007). This philopatry varies among species and perhaps regionally because of the distribution of the ponds and Smith and Green (2005) suggested that philopatric behaviour is dependent on breeding site requirements. There is a contradiction in the literature about the question whether amphibians choose or not their breeding habitat to optimize tadpoles survival (Laurila and Aho 1997, Resetarits 2005, Brönmark and Edenhamn 1994). Warren and Büttner (2007) showed the importance of habitat mosaic for amphibian diversity and rarity, providing different habitats for rare species. It seems that ponds selected for the present study are not diversified enough to find significant pond occupancy differences.

This geographical peculiarity of the Seeland results in groups of nearby ponds with high connectivity and isolated ponds with low connectivity both occupied by several species. This explain the negative effect of connectivity on diversity and on rarity. Logistic regression without this variable for *R. temporaria, B. bufo,* diversity, rarity and *R. esculenta* complex survival, which were influenced by connectivity, to see whether it was hiding the effects of other variables because of its widespread influence, couldn't prove that fish affect amphibian ponds occupancy in the Seeland (except for *B. bufo*, which was already proved with the analysis including connectivity). R. *temporaria* pond occupancy was explained significantly by *Dytiscus* sp. with a positive effect (P-value = 0.0473 *, estimate = 1.91, standard error = 9.628e-01). When connectivity was taken into account for the analysis, it was the only significant variable. Fish was the only variable explaining significantly *B. bufo* pond occupancy, with a positive effect (P-value = 0.0194 *, estimate = 2.351, standard error = 1.006). The same result occured when connectivity was considered for the analysis, but connectivity was influenced *B. bufo* pond occupancy. Forest was the explaining variable for diversity with a positive effect (P-value = 0.0183 *, estimate = 6.908e-07, standard error = 2.927e-07) and for rarity also with a positive effect (P-value = 0.0094 **, estimate = 1.086e-06, standard error = 4.183e-07). Canal proximity also explained rarity significantly, with a negative effect (P-value = 0.0239 *, estimate = -3.895e-03, standard error = 1.724e-03).

Methods

Data collection

Presence/absence is the usual method for amphibians conservation studies (MacKenzie et al. 2006). Nevertheless, it could have been interesting to take into account abundance and/or density of amphibians species to test effects of variables. For example for waterfrogs which were found in all ponds in this study, abundance could have been different between fishless and containing fish ponds, as in the study of Semlitsch (1993). He found that only 0,33% of the tadpoles survived with fish, 48,9% with newts and 70,8% with odonate larvae.

Data analysis

Detection probability was not considered for the analysis. As Mazerolle et al. (2005) wrote that many amphibians are cryptic and difficult to census. This can have serious implications. Nondetection can lead to underestimate species presence and affect inferences and conclusions about species spatial distribution and associations with patch or landscape attributes (Moilanen 2002, Gu and Swihart 2004).

The results about minimum number of visits show that nondetection may have biased some results of the present study. More visits are needed to avoid the false absence problem if detection probability is not taken into account. But readers can imagine that carrying out enough visits at every period for every species was impossible, due to logistic and field constraints. It could be interesting to reiterate this study and to use both database to have more visits per species. This would bring more complete and powerful results.

It could have been interesting to test several models combining different variables. This was done with the program PRESENCE (MacKenzie et al. 2002) which takes into account probability detection. Nevertheless, for logistic reasons, results were not available, probably because data were not sufficient for some species.

The idea to test every variable independently and not combined to built models was based on existing results showing that variables at local and landscape scales do both have important influences on amphibians occupancy (Pope et al. 2000, Bosch et al. 2006).

Conclusion

This study couldn't prove that fish really affect amphibians in the Seeland. It seems that there isn't any factor that can explain by itself amphibian distribution patterns and survival. In an equal landscape, species are distributed according to different rules, depending on their ecology and needs for habitat selection. For some species, pond characteristics are the most important ones, for others, the landscape components determine pond occupancy. And for each species, effects of variables are different. This results highlights the importance and implications to take into account the multi scale-effect of landscape processes and habitat quality for conservation (Cushman 2006), especially in the case of amphibians that are very sensible animals due to their complex life cycle. To protect core habitats, it is also of primary importance to apply conservation measures to the surrounding environment (Denoël and Lehmann 2006). But one landscape variable, pond connectivity, seem to influence amphibian distribution pattern more generally. This would mean that the metapopulation level is the most important feature enabling amphibian populations and communities to persist in a region. Metapopulation would mean positive effects of connectivity. Nevertheless, the general bad effect of ponds connectivity in this study underline the importance to maintain enough variability between breeding sites to respect the different ecological demands of different amphibian species, especially rare ones.

This study also highlights the importance of taking into account detection probability to study cryptic animals such as amphibians. But to do so, enough sampling occasions are required, which is not the case for some species in the present study (*Triturus helveticus, Hyla arborea*). It would be more productive to restrict the number of ponds to be able to visit them more often for every species and to get a more complete database. Attention should also be put on the choice of breeding sites for a next study. A stratified random sampling might minimize possible correlations between the variables taken into account to study amphibian distribution patterns and survival in the Seeland.

Acknowledgements

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