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SCIENCE DIRECT

Biological Conservation 119 (2004) 297–304

BIOLOGICAL CONSERVATION

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No evidence of general decline in an amphibian community of Southern France

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Received 28 April 2003; received in revised form 20 October 2003; accepted 31 October 2003

Abstract

We investigated long term changes in an amphibian community in the Languedoc area of Southern France by comparing results of a survey of 56 ponds made in the early 1970s with results of a survey of the same localities in 2001. Based on the frequency of new occurrences and disappearances of species in the sample ponds, there is no sign of a general decline of amphibians in this area. Most species showed non-significant variation in frequency of occurrence or had increased. "Green frogs" (*Rana perezi/ridibunda/kl. grafi*) showed clear signs of decline, but this is a local phenomenon rather than a decline at the regional scale. *Pelodytes punctatus* probably declined also, possibly due to its sensitivity to fish introduction. The species richness in the ponds has also remained stable except in ponds where fish have been introduced. The only adverse impact of human activities that we could detect was thus the introduction of fish, which had a significantly adverse effect on amphibian species richness.

Keywords: Amphibian decline; Conservation; Community; Fish; Population dynamic

1. Introduction

The decline of amphibian populations in many sites around the world has focused the interest of many researchers because, in addition to declines due to habitat modification or alien species introduction affecting many living organisms, some instances of populations decline or extinction have occurred without obvious reasons (Alford and Richards, 1999). In extreme cases, they have led to the total extinctions of species from seemingly unmodified habitats, such as the golden toad *Bufo periglenes* in Costa Rica (Pounds and Crump, 1994) or several Australian species which can no longer be located in the wild (Laurance et al., 1996). The absence of local explanations suggested some global (worldwide) phenomenon. Currently favoured explanations for these dramatic population declines that are not explained by habitat alteration include climate change, epidemic disease, chemical pollution or increase in ultraviolet radiations, (Pounds and Crump, 1994; Laurance et al., 1996; Berger et al., 1998; Alford and Richards, 1999; Pounds et al., 1999; Halliday, pers. commun.) or interaction of these factors (Kiesecker et al., 2001).

The emerging pattern of population declines depicts some differences between the main biogeographical regions of the world. Population declines apparently attributable to global phenomena have been observed in the Neotropical and Nearctic regions and in Australia (e.g., Drost and Fellers, 1996; Fisher and Shaffer, 1996; Laurance et al., 1996; Berger et al., 1998; Pounds et al., 1999; Houlahan et al., 2000; Kiesecker et al., 2001) while most European reports of amphibian declines were clearly linked to local factors such as pollution or change in land use (see Kuzmin, 1994; Alford and Richards, 1999). Furthermore, Western European amphibian communities do not appear to show a generalized downward trend since the 1960s (Houlahan et al., 2000). Nevertheless, Martínez-Solano et al. (2003) recorded significant declines of several species in

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doi:10.1016/j.biocon.2003.12.004

undisturbed habitats of Central Spain between the 1980s and 1999, partly linked with a chytrid infection.

Unlike most of Northern Europe, the Mediterranean region of Southern France, except for the coastal lowlands, has not experienced a heavy increase in industrialisation or intensification of agriculture during the 20th Century. Indeed, the most prominent feature of landscape evolution is a reduction of the proportion of cultivated or grazed lands (Lepart and Debussche, 1992; Debussche et al., 1999). Furthermore, in the Languedoc area of Southern France, ponds remain abundant as they are widely used as drinking place for livestock, and new ponds are still being created (pers. obs.). As the area is virtually devoid of intensive agriculture and industrial activities except on the littoral plains, pollution of aquatic habitats is minimal as well. This area thus offers a good opportunity to study the dynamics of amphibian communities in the absence of extensive habitat changes.

A presence/absence survey of amphibian fauna in Languedoc ponds was made between 1972 and 1974 during a study of neoteny in *Triturus helveticus* (Gabrion, 1976). We conducted a new survey of the same ponds in 2001 and compared the results from the two periods to record changes in amphibian communities between 1974 and 2001. In addition, we recorded a number of ecological variables for each pond during the 2001 survey. We first examined changes in species abundance at the regional scale by comparing the observed ratio of net extinctions and colonisations between the early 1970s and 2001 to an expected ratio of 1:1 for each species. We then investigated the causes of local changes in species richness by looking for relationships between the ecological variables of the ponds and the changes in number of species in these ponds.

2. Methods

2.1. Study area and amphibian fauna

The study area lies within the Languedoc region of southern France inside a triangle delimited by the cities of Montpellier, Millau and Alès. It is mostly within typical Mediterranean climate. Human impact is limited due to mostly extensive agriculture and sheep and cattle grazing. Furthermore, farming activities (cultivation and grazing) have been much reduced since the early 20th Century (Lepart and Debussche, 1992; Debussche et al., 1999), resulting in landscape modifications such as extension of forested area at the expense of open habitat. The inventoried ponds – except one in the littoral plain – lie within two main natural entities: the calcareous hills beyond the littoral plain, generally below 500 m a.s.l. and moderately populated, where the main human impact is now the development of residential areas and roads, and the Grands Causses, an area of calcareous plateaus between 700 and 1000 m a.s.l., with a stronger persistence of agricultural activities than in the hills area. The main amphibian breeding habitats in these areas are man-made ponds often dug out to provide drinking places for domestic livestock. Natural water bodies (mostly streams) are reasonably abundant in the low-lying hills but are much rarer in the Grands Causses area. The amphibian fauna of the whole Languedoc, including the study area, is well known due to the inventory work of amateur and professional herpetologists (Geniez and Cheylan, 1987).

2.2. Species inventories

The results of the 1970s survey consist of a list of amphibians present in 67 ponds (out of a total of 118 visited ponds) where *Triturus helveticus* had been found (Gabrion, 1976). The 1970s survey was based on repeated visits to the sample ponds between 1968 and 1974. All ponds were visited at least twice every year. Some species were lumped in the published data set and the 1970s survey details the occurrence of the following taxonomic categories: *Triturus helveticus*, *Triturus marmoratus*, *Bufo* (both *Bufo bufo* and *Bufo calamita* were found), *Pelodytes punctatus*, *Pelobates cultripes*, *Rana perezi/ridibunda/kl. grafi*(= *Rana* PRG hereafter), and "other species" (*Alytes obstetricans* + *H. meridionalis* + *Salamandra salamandra*). The green frogs were reported as *Rana temporaria*, an obvious mistake as this species is totally absent from the study area and the surrounding regions.

The survey realised in 2001 aimed at repeating the 1970s survey. We managed to visit 56 of the 67 ponds included in the 1970s survey. Some ponds had clearly disappeared, others were on private lands that we could not get permission to access, and we were unable to locate a few ponds. The 2001 survey is based on at least two visits to all the ponds. One visit was made early in the breeding season (March–April), and the other later (April–May) to maximise the chances of detecting early breeding species (*Pelodytes*, *Pelobates*, *T. helveticus* and *T. marmoratus*, *B. bufo*) and late breeding species (*Rana* PRG, *Alytes*, *Hyla*, *B. calamita*). In addition, we visited 32 ponds which had been visited during the 1970s survey but where *T. helveticus* had not been found: for these ponds we have no other information on the amphibian fauna of the 1970s.

Species occurrence in the 2001 survey was based on all observations of amphibians in or around the ponds: adults seen or heard, eggs and larvae. The pond margins and, whenever possible, most of the pond area were visited and amphibian adults, eggs or larvae looked for by visual inspection and dip-netting. Identification was mostly done in the field, but for some difficult cases (larvae of *Bufo*, young larvae of *Rana* and *Pelodytes*), samples were taken and identified in the laboratory.

There were no identification problems except for green frogs which were not identified to species (although samples have been collected for future genetic identification).

One visit was made during the day, when habitat variables were also noted (see below), while the other visit was made at night when most amphibian activity occurs. We avoided making visits when there was a strong wind or during periods of cold weather. No attempt was made to standardise survey methodology. On the contrary, we adapted the time spent on each site according to the pond area and habitat complexity. Based on our experience of repeated surveys of many similar habitats in the same area over the past ten years, this approach maximises the detection probability of the species present in the pond on any given visit. We only recorded presence/absence data.

2.3. Habitat variables

No habitat description is available for the 1970s survey, except for the presence of fish. A short written description of each pond was nevertheless provided by Gabrion (1976) but could only be used to verify that we visited the correct pond. During the 2001 survey, we recorded 22 aquatic and terrestrial habitat variables for each pond (see Table 1). Fish were detected by visual observations or by dip-netting while looking for amphibians. These techniques are not optimal for detecting fish, especially in deep and turbid ponds. Our data thus might underestimate the frequency of fish in the ponds. The fish species found in the ponds were *Gambusia affinis* in two ponds and various cyprinids (mainly *Carrassius* sp., including *Carrassius auratus* of the red domestic form, and *Tinca tinca*) in the other ponds. None of these species is native in the study area and most where introduced after the 1970s survey (see Section 4). In addition, pond size was estimated as the area of water surface at the time of the day visit. Given the small size and distance from natural water bodies of these ponds, fish presence mostly or only result of human introduction.

2.4. Data analyses

Change in a species' distribution between the two surveys was assessed using Jaccard's coefficient of similarity (number of ponds occupied during both surveys/[number of ponds occupied in the 1970s survey + number of ponds occupied in the 2001 survey − number of ponds occupied during both surveys]). This coefficient ranges from zero for a species occupying no pond in common between the two surveys (complete turnover) to 1 for a species occupying the same ponds in the two surveys (no turnover).

Table 1
List of habitat variables and coding used for the analyses

Name	Description	Coding
FOR	Presence/absence of forest in a 200-m radius	0–1
CUL	Presence/absence of cultivation in a 200-m radius	0–1
MDP	Maximum depth	1 = <0.5 m; 2 = [0.5 m, 1 m]; 3 = >1 m
TUR	Water turbidity	1 = transparent water; 2 = clear water; 3 = trouble water; 4 = turbid water
WAL	Extent of walls around the pond	0 = no wall; 1 = wall on 1 side; 2 = wall on 2 sides; 3 = wall on 3 sides; 4 = walled pond
BAR	Extent of barren shore	0 = no barren shore; 1 = 0–25% barren shore; 2 = 25–50% barren shore; 3 = 50–75% barren shore; 4 = 75–100% barren shore
HG	High grass cover in a 2 m-radius	0 = no high grass; 1 = <25% cover; 2 = 25–50% cover; 3 = 50–75% cover; 4 = 75–100% cover
SG	Short grass cover in a 2 m-radius	As *HG*
SHR	Shrub cover in a 2 m-radius	As *HG*
TRE	Tree cover in a 2 m-radius	As *HG*
ART	Artificial pond bottom	No = 0, yes = 1
CLA	Presence/absence of clay on the bottom	0–1
EAR	Presence/absence of soil on the bottom	0–1
MUD	Presence/absence of mud on the bottom	0–1
ORG	Presence/absence of coarse organic matter on the bottom	0–1
GRA	Cover of short grass on the bottom	As *HG*
FLO	Cover of floating vegetation on the surface	As *HG*
AQU	Cover of submerged aquatic vegetation on the bottom	As *HG*
EMR	Cover of emergent aquatic vegetation on the bottom	As *HG*
FAU	Use by large mammals	0 = no sign of mammals frequentation; 1 = few signs of frequentation; 2 = moderate frequentation; 3 = many signs of frequentation
FIS	Presence of fish	0–1

Changes at the regional scale (the whole study area) were examined by looking at changes in abundance for each taxonomic category. To investigate the variation of abundance of each taxonomic category between the 1970s and 2001, we compared the number of disappearances and appearances with the distribution expected under the null hypothesis of no population change. Amphibian populations in fragmented habitats (such as ponds in dry landscape) are maintained at a regional scale through a dynamic equilibrium of local extinction and colonisation. Under the null hypothesis of no population change, the numbers of extinctions and colonisations should be equal. We compared the observed numbers of disappearances and appearances for each taxonomic category with the expected numbers based on the number of local changes observed for this taxonomic category and the null hypothesis of equal numbers of disappearances and appearances. We used χ^2 tests or, when at least one observed or expected number of colonisation or extinctions was less than five, we calculated the exact probability of observing an event as likely or less likely than the observed event (sum of binomial probabilities, two tailed: sum of the probabilities of observing as many or more appearances and as many or more disappearances). For all species except *T. helveticus*, we used the 56 ponds for which a list of amphibians was available for both inventories. For *T. helveticus*, we have a sample of 56 ponds where the species was present in the 1970s and where the species can only have become extinct, and a sample of 32 ponds where the species was absent or not detected in the 1970s and where the species can only have colonised (see above). We compared the number of observed colonisations and extinctions with the numbers expected under the hypothesis of equal probability of each type of event.

Changes at the local scale refer to changes within ponds. For each pond, we computed the difference in number of taxonomic categories between the first and the second survey (*DIFNESP*). We then investigated the effects of land use, of pond surrounding habitats, of pond characteristics, of the presence of fish and of large mammals' frequentation on the variation in species number between both inventories. For land use, we made a MANOVA using *FOR* and *CUL* as qualitative predictors of *DIFNESP*. For the effect of presence of fish, we made an ANOVA using *FIS* as a predictive variable of *DIFNESP*. Effects of fish on each species were analysed by comparing the frequency of occurrence of this species in ponds with and without fish, by means of Fisher's exact tests (none of the table was suitable for χ^2 tests). To check that any significant effect is due to the introduction of fish, and not to a given amphibian species avoiding ponds where fish are more likely to be established (deep and permanent ponds), we tested whether the distribution of the species affected by fish in 2001 was also biased the 1970s by using the same group of ponds (i.e. comparing the frequency of occurrence of each amphibian species in the 1970s survey between ponds that will have fish in 2001 and those that will still be fishless in 2001). For the effect of large mammals, we made a linear regression of *DIFNESP* on *FAU*.

The effects of the habitat surrounding ponds were investigated by using *WAL*, *BAR*, *HG*, *SG*, *SHR* and *TRE* as semi-quantitative predictors of *DIFNESP* in a multiple linear regression. For the effects of the ponds characteristics, the following variables were used: *GRA*, *FLO*, *AQU* and *EMR* describe the aquatic vegetation, *TUR* and *MDP* are physical environment variables, and *ART*, *CLA*, *MUD*, *EAR* and *ORG* describe the pond substratum. As the number of variables exceeds the threshold of a tenth of the number of observations (56 ponds), we first made a PCA on these 11 variables to extract uncorrelated synthetic axes that best describe the variance of the data set. The first five axes were then entered as quantitative predictors of *DIFNESP* in several linear regressions (equivalent to a single multiple linear regression as successive axes of a PCA are uncorrelated).

3. Results

3.1. Species turnover and changes in species abundance

All species found in the 1970s survey were again observed in 2001, although *Salamandra salamandra* was not found in one of the 56 ponds that were studied in the 1970s but was recorded in a recently created pond within the study area. One additional species was found in 2001: a flourishing population of *Triturus alpestris* now inhabits one of the ponds inventoried in the 1970s (on the "Causse du Larzac"). This species is not a member of the Languedoc amphibian community and this population is known to result from a voluntary introduction in the 1980s (Geniez, pers. commun.).

Changes in amphibian community were detected in most ponds. Only five of the 56 ponds had exactly the same taxonomic categories in both surveys. There were between zero and five changes in taxonomic categories occurrence per pond (appearances or disappearances), with an average of 2.2 changes per pond. Most taxonomic categories experienced a considerable number of apparent extinction and colonisation events (Table 2). Similarity of distribution of taxonomic categories among ponds compared between surveys (measured as Jaccard's coefficient) averaged 0.37 and ranged from 0.10 to 0.67 (Table 2), indicating a considerable turnover.

In spite of this high turnover, there was little evidence for net change in abundance at the regional scale (total number of occupied ponds in the surveyed sample) for each taxonomic category between the two surveys

Table 2
Results of the 1970s and 2001 inventories, changes in the frequency of occurrence of each taxonomic category and Jaccard's coefficient of similarity

Taxonomic category or species	Number of occurrences (1970s)	Number of occurrences (2001)	Number of appearance	Number of disappearances	Significance of the trend	Jaccard's coefficient of similarity
Triturus helveticus	56[a]	54[b]	9[c]	11[c]	0.422 NS ↑	0.67
Triturus marmoratus	11	25	18	4	**0.004** ↑	0.24
Bufo	32	41	14	5	0.039 ↑	0.59
Rana	9	2	1	8	0.039 ↓	0.10
Pelobates cultripes	7	5	2	4	0.687 NS ↓	0.33
Pelodytes punctatus	35	25	10	20	0.068 NS ↓	0.33
Hyla + *Alytes* + *Salamandra*	15	39	26	2	< **0.001** ↑	0.32
Bufo bufo	–	33	–	–	–	
Bufo calamita	–	17	–	–	–	
Hyla meridionalis	–	29	–	–	–	
Alytes obstetricans	–	16	–	–	–	

Number of occurrences based on the 56 ponds inventoried both in the 1970s and in 2001 except for *Triturus helveticus* (88 ponds, see methods). Probabilities in bold are significant after sequential Bonferroni correction. Upward arrows indicate increase, downward arrows decrease.

[a] The ponds of the 1970s survey were chosen because they were all inhabited by this species (see methods).

[b] 44 ponds among the 56 ponds surveyed in the two inventories.

[c] The expected number of appearances and disappearances for this species are 7 and 13 respectively (see methods).

(Table 2). Four taxonomic categories increased in frequency, while three decreased. The only taxonomic category that shows a significant decline is *Rana* PRG, although *Pelodytes* also showed a clear decrease in number of populations, near the usual threshold for significance. The other categories show no significant trend or a significant increase (*T. marmoratus*, *Bufo* sp., *Hyla* + *Alytes*). The only significant changes after sequential Bonferroni correction (Rice, 1989) are the increases in the frequency of *Hyla* + *Alytes* and *T. marmoratus*. The average change in species richness is positive (mean $DIFNESP = 0.30$, S.D. = 1.78) but not significantly different from zero, indicating than on average we detected slightly more taxonomic categories per pond in 2001 than in the 1970s. Change in species richness ranged from −4 to 4 (Fig. 1). There is a slight but significant correlation between the number of taxonomic categories found in a pond in the 1970s and in 2001 ($p = 0.041$), indicating that species richness remains correlated over time in spite of a considerable amount of change in species richness between both inventories (adjusted $R^2 = 0.058$).

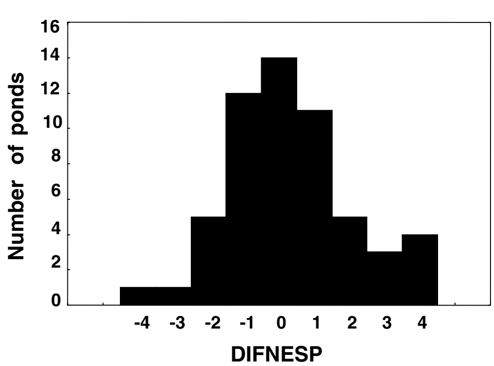

Fig. 1. Distribution of the change in number of taxonomic categories between the 1970s survey and the 2001 survey.

3.2. Factors affecting changes in local species richness

Pond size did not affect either species richness in 2001 ($p = 0.37$) or change in number of taxonomic categories between the two surveys (*DIFNESP*, $p = 0.72$). There was no significant effect of the frequentation by large mammals (*FAU*, $F_{1,54} = 2.90$, $p = 0.094$), but a significant effect of the presence of fish (*FIS*, $F_{1,54} = 9.13$, $p = 0.004$, see Fig. 2), which were found in 25% of the 56 inventoried ponds. On average, fishless ponds gained in species richness between the two surveys (mean $DIFNESP = 0.69$) while species richness decreased in ponds with fish (mean $DIFNESP = -0.86$). *Pelodytes punctatus* was the most affected by the presence of fish: it was found in 2001 in more than half of the fishless ponds (23 out of

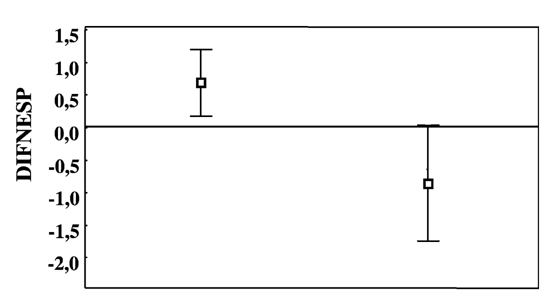

Fig. 2. Difference in number of taxonomic categories between the 1970s and 2001 in ponds with and without fish.

Table 3
Correlations of the variables with the first five axes (PC1 to PC5) of the PCA on 11 aquatic habitat variables

Variables	PC1	PC2	PC3	PC4	PC5
MDP	0.015	0.310	−0.401	0.613	0.181
TUR	0.264	−0.144	0.057	0.871	0.034
ART	0.868	0.261	−0.194	−0.250	−0.071
CLA	−0.247	−0.447	0.730	0.150	0.342
EAR	−0.735	0.131	−0.464	0.151	−0.217
MUD	0.133	0.706	0.231	0.017	−0.255
ORG	−0.105	0.716	0.431	−0.075	−0.130
GRA	−0.683	−0.282	−0.126	−0.166	−0.425
FLO	−0.130	0.123	0.684	0.187	−0.334
AQU	−0.388	0.357	0.005	−0.345	0.675
EMR	−0.447	0.591	−0.039	0.242	0.172

42) but only two of the 14 ponds with fish (Fisher's exact test, $p = 0.012$). No difference in frequency of occurrence of this species between the ponds with fish in 2001 and the ponds without fish in 2001 could be detected in the 1970s data, i.e. before fish introduction ($p = 0.532$). This might explain why this species decreased in our surveyed ponds between both inventories. Newts were affected also, as *T. helveticus* disappeared from seven of the 14 ponds where fish has been stocked, but only from four of the 42 fishless ponds (Fisher's exact test, $p = 0.028$ As this species was present in all these ponds in the 1970s this effect measures directly the effect of fish on extinction probability. *T. marmoratus* was present in 2001 in 22 from the 42 fishless ponds but in only three of the 14 ponds with fish (Fisher's exact test, $p = 0.063$). When comparing frequency of occurrence between the same groups of ponds before fish introduction, no bias was apparent ($p = 0.711$). Other species might have been affected also but the low number of ponds with fish prevented from detecting significant trends: *B. calamita* and *A. obstetricans* were found in only two ponds with fish, while they occurred in 15 of the 42 fishless ponds (Fisher's exact test, $p = 0.186$). Only *Hyla meridionalis* and *B. bufo* were truly not adversely affected by fish (*Hyla* was present in half of the fishless ponds and 8 of the 14 ponds with fish, *B. bufo* in 23 of the 42 fishless ponds and 10 of the 14 ponds with fish). *Pelobates cultripes* and *Rana* PRG were too scarce to be examined for this factor.

There was no effect of the land use (presence of forest or cultivated land around the ponds) on the change in species richness ($DIFNESP = FOR + CUL + FOR*CUL$, $F_{3,52} = 0.40,\ p = 0.752$) but there was a significant effect of the surrounding habitat of the pond (extent of wall, barren shore, high grass, short grass, shrubs and trees) ($DIFNESP = WAL + BAR + HG + SG + SHR + TRE$, $F_{6,49} = 3.37,\ p = 0.007$) although this model only explained about 20% of the variance (adjusted $R^2 = 0.205$). The only significant effects were due to negative effects of the extent of barren shore (*BAR*, partial regression coefficient $\beta = -0.79,\ p = 0.021$) and of shrubs (*SHR*, $\beta = -0.48,\ p = 0.037$). None of these were significant when *BAR* or *SHR* were entered alone in a simple linear regression. This was probably due to a significant negative correlation between *BAR* and *SHR* ($R = -0.52,\ p < 0.001$).

There was no effect of the first, second, third and fifth axes of the PCA of aquatic habitat variables, but there was a moderately significant effect of the fourth axis on *DIFNESP* (adjusted $R^2 = 0.072$, $F_{1,54} = 5.24,\ p = 0.026$). The variable contributing most to this fourth axis are *TUR* and *MDP* on the positive side (see Table 3); this axis thus separates deep and turbid ponds from the other ponds. As ponds that are on the positive side of the fourth axis are also more likely to be inhabited by fish ($PC4 = FIS$, $F_{1,54} = 15.11,\ p < 0.001$), we entered *FIS* and *PC4* together in a Generalized Linear Model (*DIFNESP = PC4 + FIS*). The effect of *PC4* is not significant any more; the effects of the aquatic habitat variables on the change in species number thus seem to be explained by the effect of fish, which are significantly more often found in deep and turbid ponds.

4. Discussion

4.1. Quality of the data in the 1970s survey

The misidentification of the green frogs in the 1970s survey and the lumping of several species in one taxonomic category cast doubts on the validity of the species identification in the older dataset. The lumping of *A. obstetricans*, *S. salamandra* and *H. meridionalis* had nothing to do with identification problems, however, but was for practical space reasons in the tables, as both *Salamandra* and *Alytes* were very rare in the 1970s survey (Gabrion, pers. commun.). Lumping of *B. bufo* and *B. calamita*, on the other hand, resulted from the difficulties in identifying larvae of these species. As the 1970s inventory lists all the species known to occur or to have occurred in the area at this time (based on independent regional and national inventories), no species could have been totally missed in the 1970s inventory because of misidentification.

A further uncertainty is the efficiency of detection of species in the 1970s survey. Pond survey in the 1970s was not aimed at producing a complete survey of amphibian species in each pond and detection probability on a per visit basis was thus probably lower than in the 2001 survey. Nevertheless, this lower probability of detection per visit is likely to have been compensated by a much higher number of visits in the 1970s. Our own experience of surveying ponds in this area over the past 15 years has showed that variation in abundance between visits has a much greater impact on detection probabilities than sampling effort on a given visit. Recent results by Skelly et al. (2003) confirm the importance of the duration of resurvey in two-surveys comparisons and suggests that resurvey based on one year only are likely to yield an estimated decline. Thus, it is extremely unlikely that overall probabilities of species detection were higher in the 2001 survey, and that this biased our results (see below).

4.2. Lack of negative trend at the regional scale

At the species level, one taxonomic category showed a significant decline (the green frogs, *Rana* PRG) and another (*Pelodytes punctatus*) a near significant decline. None of these declines are catastrophic as these species are still abundant in the study area (pers. obs.). The frequency of occurrence of all other taxonomic categories remained stable or showed signed of increase. Due to some imprecision in the 1970s data (merging of *H. meridionalis* and *A. obstetricans*, merging of *B. calamita* and *B. bufo*), there might have been some changes in the relative proportion of *Hyla* and *Alytes* on one hand, and *B. calamita* and *B. bufo* on the other hand, but since these four species are still common in the inventoried ponds, none could have declined dramatically. *S. salamandra* was found in the 1970s in the inventoried ponds, but was qualified as "very rare" (Gabrion, 1976). We did not find it in 2001 in the inventoried ponds but we found it in one recently created pond at the north-west edge of the study area. We know from the ongoing distribution atlas of reptiles and amphibians in the Languedoc area (Geniez and Cheylan, 1987 and pers. com.) that *S. salamandra* is abundant north-west of the study area but reaches its distribution limit in the calcareous hills north of Montpellier. It is thus a marginal species in the study area, where it was very rare in the 1970s and is still very rare today. One potentially important factor which we did not study is the abundance of ponds at the regional scale. Our impression gained during several years of monitoring ponds in the area is nevertheless that there has not been a dramatic change in breeding habitats availability. While a limited number of ponds had clearly disappeared between the 1970s and 2001 (see Section 2), many ponds that exist today were not included in the 1970s survey, and we have witnessed the creation of several new ponds in the area in the last ten years. We could thus not detect any negative trend for the amphibian community at the regional scale. Even is efficiency of detection of species was higher in the 2001 survey (but see above), this could have only compensated for a limited decrease in species richness or in frequency of a given species. We can thus safely conclude that no dramatic decline similar to what have been documented in other areas of the world has occurred in our study area.

We even detected some apparently genuine instance of increase between the two surveys. As *T. marmoratus* spends long period of time in the water as adults and larvae and since the visits of the 1970s survey were aimed at finding the related but easily distinguished *T. helveticus*, *T. marmoratus* is probably the species which is less likely to have been under recorded in the 1970s survey, and its marked increase is likely a real phenomenon. *A. obstetricans* was not found in the Causses area in the 1970s survey except in one pond (C. Gabrion, pers. commun.) while it was found in 2001 in many ponds of the Causses. The colonisation of the Causses habitat by *Alytes* between the 1970s and 2001 is probably the reason for the marked increase of the *Hyla* + *Alytes* taxonomic category.

4.3. Species turnover and adverse factors at the local scale

Species turnover per pond and changes in species distributions for all species indicate that the stability of the amphibian populations at the regional scale is achieved through numerous cases of local extinction and colonisation. Number of extinctions and colonisations are probably underestimated in our data, because we do not know how many extinctions and colonisations have actually taken place between both surveys for a given situation. This pattern of equilibrium at the regional scale maintained by a dynamic process of extinction–colonisation cycles at the local scale is typical of metapopulation models (Hanski, 1999), which is thought to apply to many populations of aquatic-breeding amphibians (Marsh and Trenham, 2001; Semlitsch, 2002 , but see also Bradford et al., 2003). A high level of population turnover has indeed been documented in a North-American study following a protocol similar to our approach (Skelly et al., 1999).

There is no decrease in the average number of taxonomic categories per pond between the 1970s and 2001, but some ponds had lower species richness in 2001 than in the 1970s. The effects of terrestrial habitat variables on variation in species richness are difficult to interpret (see results) and will not be further discussed here. Among the aquatic habitat variables, only the presence of fish have a significant impact on the change in species richness over the past 30 years. As fish were absent in the 1970s from the studied ponds except for *Gambusia* in a single pond, effects of the presence of fish on species richness can thus be interpreted as a direct effect of fish introduction: introducing fish to a pond led in average to a decrease in species richness in this pond.

Based on census data, effects of fish on occurrence of individual species are difficult to interpret due to possible confounding variables. For *T. helveticus*, which was present in all the ponds from the 1970s survey, the introduction of fish can be interpreted in term of extinction probability, so for this species we have a real measure of the impact of fish. For the other species that we found to be significantly less frequent in ponds with fish, the fact that they did not avoid these ponds before the introduction of fish indicates that they were negatively affected by fish introduction itself.

There is already an extensive amount of empirical work demonstrating the negative impact of fish on the abundance of some amphibian species (e.g., Aronsson and Stenson, 1995; Knapp and Matthews, 2000; Gillespie, 2001; Nyström et al., 2002) or on amphibian communities (e.g., Braña et al., 1996; Hecnar and M'Closkey, 1998; Smith et al., 1999). In the ponds of the Languedoc area, fish introduction seems to be the main negative human impact on amphibian populations.

Acknowledgements

We are grateful to P. David, T. Halliday, J.-D. Lebreton, R. Prodon and an anonymous referee for valuable comments on previous versions of the manuscript and to P. Geniez for general assistance with this study. J. and C. Gabrion kindly accepted to answer our queries on the 1970s survey.

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