Inter-annual variability in amphibian assemblages: implications for diversity assessment and conservation

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ABSTRACT

1. Diversity assessments and conservation management should take into account the dynamic nature of populations and communities, particularly when they are subject to highly variable and unpredictable environmental conditions.

2. This study evaluates the inter-annual variability in the assemblage composition (temporal turnover) of an amphibian community breeding in a highly dynamic habitat, a Mediterranean temporary pond system, during a 4-year period.

3. A comprehensive framework is provided to evaluate temporal turnover from data of a differing nature (species richness, presence/absence and relative abundance) and, especially, to discern variation in richness (species loss) from changes in the identity or abundance of species (species replacement).

4. Results show that the pond amphibian assemblages in Doñana National Park exhibited high inter-annual variability during the study period, both in the number of species, species identity and their relative abundance. This result provides evidence for the inadequacy of surveys conducted only in one breeding season to characterize the species assemblage associated with a given pond. Besides, it suggests that a given pond offers different breeding opportunities over time, being suitable for different species depending on the year. This alternation will contribute to the medium-term preservation of all species in the assemblage.

5. It is highly relevant to preserve the natural dynamism and spatial variability of temporary pond systems, which will favour the conservation of populations through their intrinsic variability.
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INTRODUCTION

Stable ecosystems, defined as systems with no temporal variation, are more the exception than the rule (Ricklefs and Schluter, 1993). In fact, it is widely accepted that both habitats and biological communities may vary over ecological time (Preston, 1960; Collins and Glenn, 1991; Fjeldsa and Lovett, 1997; Innes, 1998; Magurran, 2007), which stresses the claim for a temporal perspective in ecological studies, especially in the analysis of biodiversity patterns (Buckland *et al.*, 2005; Magurran, 2007). For this reason, the dynamic nature of populations and communities has long been a subject of interest *per se*. For instance, ecologists have aimed to evaluate the temporal variation in diversity (Skelly *et al.*, 1999; Werner *et al.*, 2007; Azeria and Kolasa, 2008), to disentangle natural population fluctuations from declining trends that might compromise the preservation of the species (Green, 2003; Loman and Andersson, 2007) or to evaluate causes of variation in assemblage composition, such as local habitat changes (Briers and Warren, 2000; Biedermann, 2004) or stochastic extinction-colonization events in a metapopulation scenario (Hels, 2002; MacKenzie *et al.*, 2003). Understanding the temporal variability in ecological systems is also crucial for successful management and conservation, which depends on the recognition of their dynamic nature (Hobbs, 1998). From a practical standpoint, the temporal dynamism of species

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assemblages may become an error source in biodiversity assessments (i.e. spatial distribution of species) and those ecological interpretations derived from them. For instance, not taking into account the natural variability of communities may lead to wrong conclusions about the conservation status of a species and/or the temporal trends of its populations. In order to avoid these flawed data in dynamic systems, it is necessary to evaluate whether species assemblages actually change over time.

Many amphibian communities are highly variable over time, with changes in the number or abundance of species from year to year (Pechmann *et al.*, 1991; Hecnar and M'Closkey, 1996; Skelly *et al.*, 1999; Trenham *et al.*, 2003; Werner *et al.*, 2007). At a regional scale, these changes may be interpreted as intrinsic natural fluctuations in population size (Marsh, 2001; Pellet *et al.*, 2006) which may also uncover trends in population size (i.e. decline) critical for species conservation (Green, 2003; Collins and Halliday, 2005). At pond scale, year-to-year changes in species occupancy may be due to extinction–colonization events (Skelly *et al.*, 1999; Richter-Boix *et al.*, 2007; Werner *et al.*, 2007), regular inter-pond movements of individuals (Marsh and Trenham, 2001) or no breeding activity. If individuals do not breed or move to an adjacent pond, the species is still occupying the same breeding unit and hence the absence from that particular pond should be interpreted as a temporary event, probably in response to temporary changes in habitat suitability. In other words, the population size does not change and, therefore, changes in the occupancy of a species due to these occasional events do not compromise the conservation status of the species. However, it should be noted that lack of breeding activity during several and consecutive years may be detrimental for the species. Several studies have reported that temporal variation in amphibian assemblages results from inter-annual variability in meteorological/hydrological conditions (Jakob *et al.*, 2003) or in local habitat attributes, both in breeding ponds (Skelly and Meir, 1997) and in surrounding uplands (Skelly, 2001). In general, high temporal turnover (i.e. temporal variation in assemblage composition) is expected in non-stable habitats (Moreno and Halffter, 2001), such as Mediterranean temporary ponds.

From an applied point of view, methodological procedures appropriate for the detection and quantification of temporal turnover are necessary to avoid biases in biodiversity assessments and in evaluations of species conservation status. Temporal turnover can be assessed from data of a differing nature (i.e. presence/absence or their summary metrics, like species richness). Presence/absence is an information-poor but cheap source of data to infer community changes (Henry *et al.*, 2008). Previous studies have considered the variation in the size of breeding populations (Pechmann *et al.*, 1991), differences in species richness values (Hecnar and M'Closkey, 1996; Werner *et al.*, 2007) or dissimilarity in assemblage composition between consecutive years (Skelly *et al.*, 1999; Werner *et al.*, 2007). The first approach focuses on temporal variation at the species level, whereas the latter two summarize the variation of all species, thus providing measures of turnover at the assemblage/community level. The variation in assemblage composition can be caused either by the gain or loss of some species or by the replacement of some species by others ('true' species turnover). Comparisons of species richness values are intended to reveal changes due to gains or losses of species. They will prove misleading, therefore, if the number of species does not change but the identity does, as may occur if some species are replaced by others (Hecnar and M'Closkey, 1996). Besides, differences in sampling effort may lead to potential biases in the assessment of temporal turnover due to the fact that the probability of detecting a species increases with the accumulation of sampling effort (Gotelli and Colwell, 2001). To detect temporal turnover due to the replacement of species, comparisons should be made of the specific assemblage composition using dissimilarity indexes independent of richness values (Koleff *et al.*, 2003; Baselga *et al.*, 2007; Baselga, 2010). Therefore, this approach will provide information complementary to richness comparisons. Moreover, temporal turnover due to changes in species dominance patterns (common species versus rare species) can be evaluated also by using quantitative data (i.e. relative abundance), which is usually preferred in diversity assessments (Magurran, 2004).

Doñana National Park is one of the largest and most important wetlands of Europe. It is located in south-western Spain, eastwards from the mouth of the Guadalquivir River. It was granted the status of Biosphere Reserve by UNESCO in 1980, an Internationally Important Wetland Site under the Ramsar Convention in 1982, a Special Protection Area for Birds in 1988, and a Natural World Heritage Site in 1994. Despite being under such high protection status, it entered the Montreux Record of Ramsar sites that face threats to conservation in 1990. Doñana National Park preserves a large system of temporary ponds (Gómez-Rodríguez, 2009), a priority habitat under the European Union Habitats Directive (European Commission, 2007), with a spatial configuration conferring robustness to inter-annual variability in hydrological conditions (Fortuna *et al.*, 2006). These ponds are a critical habitat of many species of aquatic flora and fauna: macrophytes (García Murillo *et al.*, 2006), invertebrates (Bigot and Marazanof, 1966; Millán *et al.*, 2005; Serrano and Fahd, 2005), and amphibians (Díaz-Paniagua, 1990; Díaz-Paniagua *et al.*, 2005). The area has been designated as a location of 'special interest for the conservation' of amphibians (Santos *et al.*, 1996). All species from the south west of the Iberian Peninsula breed in this area except *Salamandra salamandra* (Linnaeus, 1758), a species that, in south Spain, is associated with high-altitude ecosystems. Doñana National Park provides a model system to evaluate the natural temporal dynamism of amphibian communities since it is a highly protected area. Moreover, areas with such a high density and heterogeneity of natural ponds in good conservation status are not common in Europe, where the number of temporary ponds are probably a mere fraction of what they would naturally have been in the past (Williams *et al.*, 2001).

This study evaluates temporal turnover in a highly dynamic ecosystem, the amphibian community breeding in Mediterranean temporary ponds in Doñana National Park. A comprehensive framework is applied to assess changes both at species and assemblage levels, and to discern variation in richness (species loss) from changes in the identity or abundance of species (species replacement). The applied interest of this study is to evaluate the adequacy of single-year surveys in amphibian diversity studies in Mediterranean systems, even when the applied sampling effort proves sufficient to detect all species breeding that season.

MATERIAL AND METHODS

Study area

The study was conducted in an area of 6794 ha within Doñana National Park (Figure 1(a)), in south-western Spain (see Siljeström *et al.*, 1994 for a geomorphological description). The dominant vegetation in this area is Mediterranean scrub (*Halimio halimifolii–Stauracanthetum genistoides* and *Erico scopariae–Ulicetum australis* as defined by Rivas-Martínez *et al.*, 1980) and isolated patches of pine (*Pinus pinea* L.) and juniper forests (*Juniperus phoenicea* L.).

On the sandy area of the Park, many temporary ponds flood during the rainy season (see Gómez-Rodríguez *et al.*, 2008). These ponds are fed by fresh water and have no direct connection to the sea. The duration of flooding (or hydroperiod) varies among ponds, from pools persisting one month to ponds persisting up to 10 months in very wet years. Annual hydroperiod is also widely variable, depending on rainfall. In years of scarce rainfall, most ponds may not flood, while hydroperiod is notably reduced in those that do flood. The area also includes two semi-permanent ponds that occasionally dry out in years of severe drought.

For this study, the two semi-permanent ponds and 19 temporary ponds (Figure 1(b)), covering the wide hydroperiod gradient in the study area, were selected (a detailed description of the temporary ponds can be found in Gómez-Rodríguez *et al.*, 2009). The pattern and amount of rainfall varied among the years of study (Figure 2) and consequently the onset and duration of surface water in ponds.

Amphibian sampling

An intensive monthly survey was conducted each year during the amphibian breeding season: February–May 2003, January–May 2004 and March–May 2006. Previous studies reported that these months comprise an adequate period of time for detecting all species in the study area (Díaz-Paniagua *et al.*, 2005). Ponds did not flood in 2005 and hence were not sampled. Some ponds could not be sampled in particular years: two ponds were not accessible in 2004 and two different ponds were flooded for less than one month in 2006. In 2003, one pond was only accessible in May.

Dip-netting techniques (Heyer *et al.*, 1994) were used to collect and identify larvae to species level *in situ* (described here as 'larval sampling'). The number of larvae captured in each sampling unit (three consecutive sweeps on a stretch approximately 1.5 m long) was counted before releasing the larvae in the pond. For most ponds, 12 sampling units were set as the standard sampling effort. Sampling units were separated a minimum of 5 m to avoid interference between surveys. Small ponds were sampled in proportion to their size, so the number of sampling units could decrease to guarantee the minimum separation distance. In large ponds, the number of sampling units was increased in order to sample all different microhabitats.
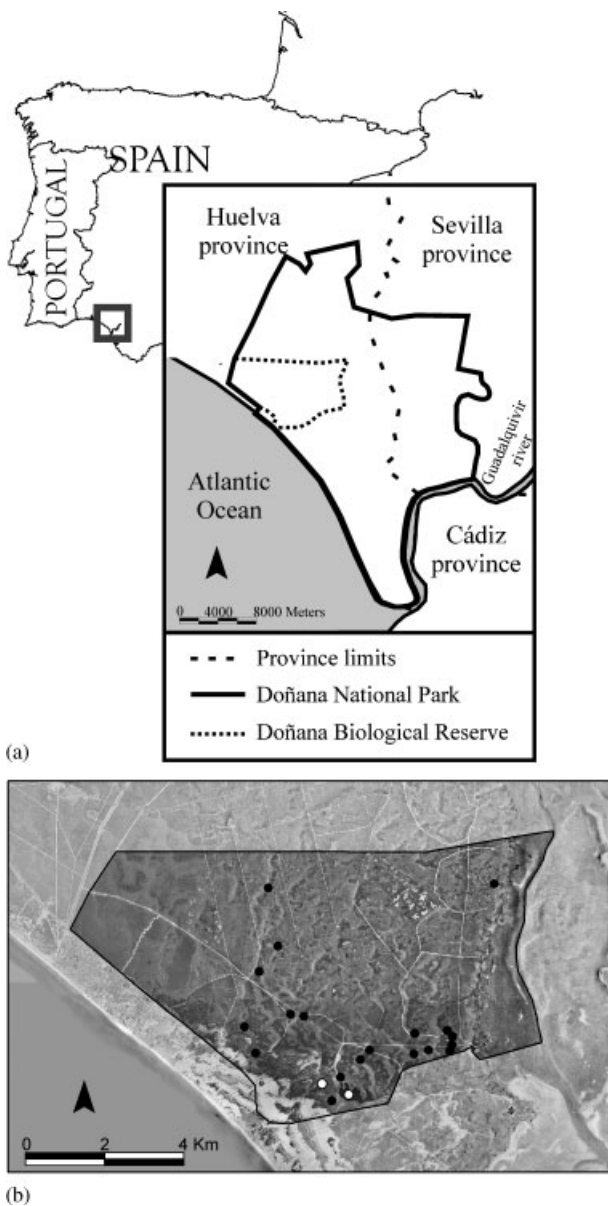

Figure 1. (a) Location of Doñana National Park in south-western Spain and (b) orthophotography of the study area. Solid line delimits Doñana Biological Reserve, and dots show the location of semi-permanent (white dots) and temporary (black dots) study ponds.
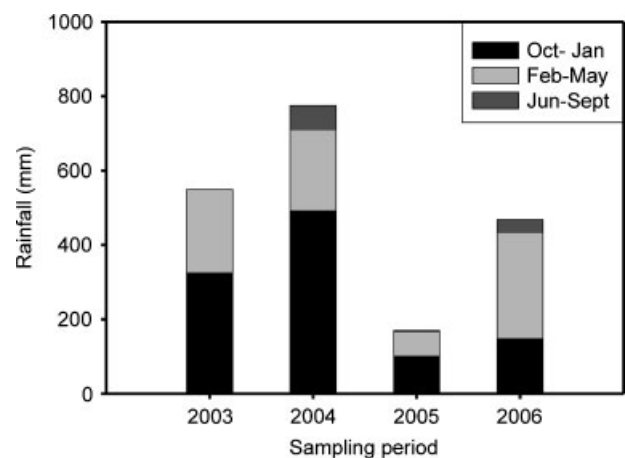

Figure 2. Rainfall input in hydrological years from 2002/2003 to 2005/2006. Three different periods were differentiated: October–January; February–May; June–September.

Larval sampling was complemented with visual surveys in and around the ponds to detect eggs, larvae and metamorphic individuals. Visual surveys were conducted regularly, starting when ponds flooded (November 2002, November 2003 and January 2006).

Data analysis

Species level

Inter-annual changes in the occupancy (proportion of occupied ponds) and overall relative abundance of species are described (see Table 1 for terminology used in this study). For each species, overall relative abundance was measured as catch-per-unit effort (number of larvae collected per total number of sampling units) in the ponds it occupied, thus excluding the ponds where the species was not recorded, in order to achieve independence with occupancy metrics. Metrics were computed both for annual fauna (data collected in each breeding season) and cumulative fauna (data collected over the entire study period).

Assemblage level

Two different approaches are provided to evaluate temporal turnover depending on the type of data available: summary metrics of pond assemblage composition (species richness) and specific pond assemblage composition data (presence or relative abundance data).

(a) Richness-based approach: to evaluate year-to-year variation in the number of species detected in a pond, a repeated measures ANOVA was computed for the annual

Table 1. Definition of biodiversity terms used throughout the text

Terminology	Definition
Species level	
Species occupancy	Proportion of ponds in which the species was detected. Depending on the period of time considered, this refers to annual occupancy (in an annual survey) or cumulative occupancy (over the entire study period)
Overall relative abundance (study area)	For each species, the ratio between the number of detected larvae and the number of sampling units in a given period (i.e. annual survey (annual) or over the entire study period (cumulative). Only the number of sampling units conducted in those occupied ponds is considered. The metric is computed from 'larval sampling'
Relative abundance (pond)	Idem for each pond
Assemblage level	
Annual richness (S_{ann})	Number of species detected in a given pond in a particular year
Cumulative richness (S_{cum})	Total number of species detected in a given pond over the entire study period
Rarefied cumulative richness (S_r)	Estimated number of species that would have been detected in a given pond with the sampling effort applied in a given season if all the species detected throughout the entire study period had been present that season (only for annual surveys)
Inter-annual/ temporal turnover	Changes in the number, identity or relative abundance of species of a defined pond assemblage from year to year

richness values (i.e. S_{ann_2003} vs S_{ann_2004} vs S_{ann_2006}; where subscript numbers refer to seasons) with posthoc Tukey test using Statistica Software. Thereafter, to evaluate temporal turnover, the procedure that Romanuk and Kolasa (2001) recommended was followed. For each breeding season, annual richness (S_{ann_2003}; S_{ann_2004} or S_{ann_2006}) was compared with the total number of species detected in that pond over the entire study period, cumulative richness (S_{cum}). This approach outperforms annual comparisons, which might be biased from changes in species identity although not in species counts (Hecnar and M'Closkey, 1996). Repeated measures ANOVA were applied to each comparison (i.e. S_{ann_2003} vs S_{cum}; S_{ann_2004} vs S_{cum}; S_{ann_2006} vs S_{cum}) and posterior Bonferroni correction to the level of significance.

This approach to detect temporal turnover does not account for differences in sampling effort and hence can be subject to potential biases due to the fact that the probability of detecting a species increases with the accumulation of sampling effort (Gotelli and Colwell, 2001). For that reason, each analysis was complemented by an additional comparison between the value of annual richness for each pond and year and its respective rarefied value, S_r, with a repeated measures ANOVA using Bonferroni correction. Rarefied cumulative richness (S_r) is an estimated value of S_{cum} correcting for sampling effort and was assessed from sample-based rarefaction curves (Gotelli and Colwell, 2001) computed from the complete dataset (2003–2006) with EstimateS 7.0 software (Colwell et al., 2004). Thus, for each sampling season, S_r estimated the number of species that would have been detected in a given pond, with the sampling effort applied that season, in the absence of inter-annual turnover (i.e. all species collected during the entire study period were detectable that season). In this analysis, annual and rarefied richness were computed from 'larval sampling' data.

(b) Assemblage-composition-based approach: traditional similarity measures, usually focused on the description of spatial variation in diversity (see Magurran, 2004), were applied to test if the composition of amphibian assemblages varied inter-annually. To determine the existence of inter-annual turnover in assemblage composition, the antagonist pattern was searched: a high similarity in composition among the species assemblages of a given pond in different breeding seasons. That is to say, to reject the hypothesis of temporal turnover, assemblages of the same pond in different breeding seasons should be highly similar, being grouped together and differentiated from assemblages of other ponds. Two sets of analyses were conducted to calculate similarity in assemblage composition: one based on presence–absence data and the other one based on relative abundance of each species, measured as catch-per-unit-effort. For each data type (presence–absence vs relative abundance), a dissimilarity matrix was computed considering as independent cases the assemblages of a given pond in different years. The Simpson dissimilarity index was used for presence–absence data and the Morisita–Horn dissimilarity index for relative abundance data because these indices are not influenced by species richness gradients and sample size (Magurran, 2004). So, both indices measure differences in species composition attributable only to true species turnover (i.e. some species are replaced by others) and not to species loss (i.e. some species disappear from the community or are not detected) (see Baselga et al., 2007; Baselga, 2010).

To evaluate temporal turnover from dissimilarity among species assemblages, an analysis of similarities (ANOSIM) (Clarke, 1993) (command anosim, package vegan, R statistical package) was computed to each assemblage dissimilarity matrix using pond identity as the grouping factor. ANOSIM evaluates whether there is a significant difference between groups of ponds, i.e. the assemblages detected in a given pond over time constitute an identifiable group. In the absence of inter-annual turnover, the ANOSIM statistic (R) would be significant and close to 1, indicating that differences among years in assemblage composition in any particular pond were much lower than differences from other pond assemblages in any year. On the same basis, an assessment was made of whether particular species assemblages were favoured in particular years (year-specific effect) by applying an ANOSIM analysis with 'year' as the grouping factor. So, if the ANOSIM statistic (R) was significant and close to 1, it would show that assemblages of different ponds measured in the same breeding season were highly similar, being grouped together and differentiated from assemblages measured in other breeding seasons. To visually clarify the interpretation of ANOSIM tests, two unconstrained ordinations of ponds using non-metric multidimensional scaling (NMDS) were conducted, one derived from each dissimilarity matrix (presence/absence vs relative abundance) using Statistica software. For each ordination, two axes were represented and the cases corresponding to each group (pond identity or year) were marked. In ordination plots, cases corresponding to the same pond would appear aggregated under low temporal turnover.

RESULTS

Species level

Nine species were identified over the entire study period: *Bufo bufo* (Linnaeus, 1758), *Bufo calamita* Laurenti, 1768, *Pelobates cultripes* (Cuvier, 1829), *Discoglossus galganoi* Capula, Nascetti, Lanza, Bullini and Crespo, 1985, *Pelophylax perezi* (Seoane, 1885), *Hyla meridionalis* Boettger, 1874, *Pleurodeles waltl* Michahelles, 1830, *Lissotriton boscai* (Lataste, 1879) and *Triturus pygmaeus* (Wolterstorff, 1905) (Figure 3). All species but *B. bufo* were detected in every breeding season (always excluding 2005, when ponds did not flood and amphibian species did not breed in the study ponds). Amphibian species exhibited marked inter-annual variation in their occupancy and overall relative abundance (Figure 3). Over the entire study period, most species showed a large occupancy. However, the proportion of ponds a given species occupied every breeding season was moderate or low for all the species. For instance, *T. pygmaeus*, the species with a higher tendency of recurrent breeding in the same pond, was detected in only half the ponds every breeding season. The two species with largest occupancy over the entire study period, *T. pygmaeus* and *H. meridionalis*, bred in more than 90% of ponds. These species also exhibited the largest annual occupancy in 2003 and 2004, although with a notable decrease in 2006. The same decrease in the occupancy in 2006 was found in the other two urodeles (*L. boscai* and *P. waltl*). *Pelobates cultripes* was also a common species, occurring in 86% of study ponds, but unlike the previous species its largest occupancy was observed in

2006. *Bufo calamita* and *D. galganoi* also occupied a larger proportion of ponds in 2006 than in 2003 or 2004. The rarest species was *B. bufo*, which is not a common breeder in temporary ponds, and was only detected, as eggs, in a semi-permanent pond in 2006. *P. perezi* was found in five ponds in 2003, not being collected in any different one in the rest of the seasons.

The pattern of overall relative abundance was largely different from year-to-year (Figure 3). The relative abundance of *B. bufo* could not be measured since it was not detected in its larval stage but only as eggs. The highest values of overall relative abundance were observed in 2006 for all species except *T. pygmaeus* and *P. perezi*, with highest relative abundance in 2003, and *L. boscai*, with highest relative abundance in 2004, although similar to relative abundance in 2006.

Assemblage level

Inter-annual turnover in pond assemblage composition was evidenced when using the richness approach. Inter-annual differences in species richness values (RM ANOVA, $F_{2,32} = 4.875$; $P = 0.014$) were observed since annual richness in 2003 was significantly higher than in the rest of the seasons (Tukey test, $P < 0.050$) while richness in 2004 and 2006 did not differ significantly (Tukey test, $P = 0.970$). However, temporal turnover cannot be inferred by comparing only annual values; a comparison between annual and cumulative values is needed. The number of species detected over the entire study period (S_{cum}) was higher than any annual richness (Table 2), indicating, for example, that some species absent in 2003 were recorded in 2004 or 2006. Accumulation of sampling effort during the entire sampling period did not explain differences between S_{cum} and S_{ann_2003} or between S_{cum} and S_{ann_2004}, because S_r was significantly higher than the annual values (Table 2). Therefore, a potential bias caused by differences in sampling effort can be discarded, provided that the number of sampling units used to compute the cumulative values were much larger than those used to compute the annual values. On the contrary, S_{r_2006} did not differ from S_{ann_2006}, suggesting that the smaller number of species detected in 2006, compared with cumulative richness, could be explained either by lower sampling effort or temporal turnover in amphibian pond assemblages.

Inter-annual turnover in pond assemblage composition was confirmed in the composition-based approach, both with presence–absence data (ANOSIM $R = 0.133$; $P = 0.024$) and relative abundance data (ANOSIM $R = 0.146$; $P = 0.008$). Despite being significant, the R statistic should be high to be relevant (Clarke and Warwick, 2001) and hence assume that ponds can be grouped based on the factor of interest (pond identity), which would imply high similarity in assemblage composition. Consequently, ANOSIM results show inter-annual temporal turnover in pond assemblage composition. In fact, the low value of ANOSIM R shows that the assemblage composition in a given pond in a particular year was only slightly more similar to the assemblage composition in the same pond in a different year than to any assemblage composition in a different pond (Figure 4(c) and (d)). Similarly, pond assemblage composition was not determined by a year-specific effect, as evidenced by the low values of ANOSIM R obtained for presence–absence data (ANOSIM $R = 0.167$; $P < 0.001$) or relative abundance data (ANOSIM
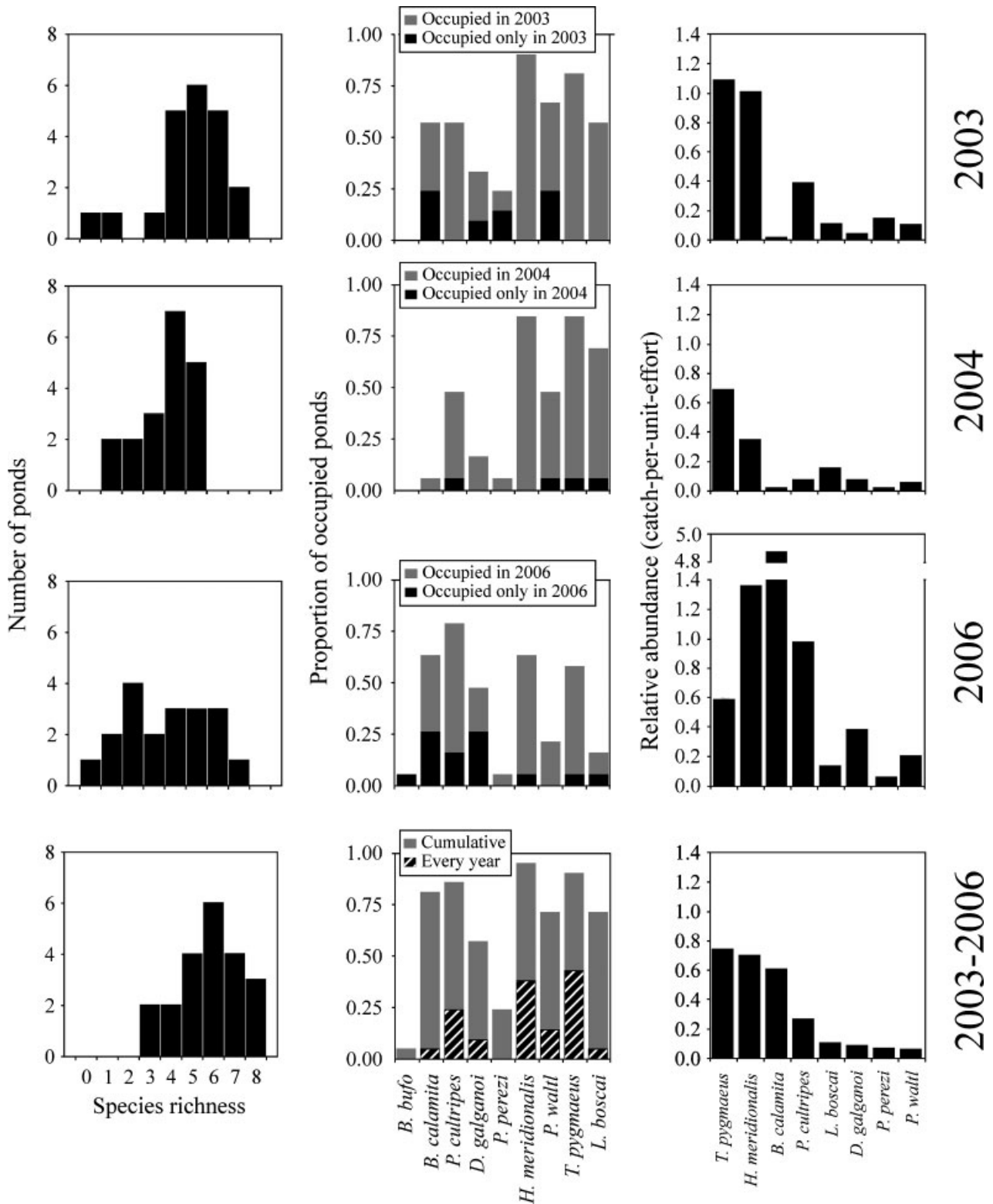
Figure 3. Species richness histograms, proportion of ponds each species occupied (occupancy) and their overall relative abundances in each breeding season and over the entire study period (cumulative data). In the proportion of occupied ponds, the proportion corresponding to the ponds in which the species was detected only in that particular year is highlighted for annual data. For cumulative data, the proportion of ponds that were used every year is also shown.

$R = 0.161$; $P < 0.001$). However, the graphical representation of the NMDS axes derived from dissimilarity matrices showed a tendency for some similarity among species assemblages in the same breeding season, as they appeared aggregated although scattered among pond assemblages of different seasons (Figure 4(a) and (b)).

DISCUSSION

The amphibian assemblages in Doñana National Park exhibited high inter-annual variability during the study period. The most dramatic change occurred in 2005, when temporary ponds did not flood due to a severe drought and hence amphibian breeding was not detected. This is an extreme case of temporal turnover due to a lack of breeding habitats. However, if only 'true' breeding seasons, when species did reproduce, are considered, temporal turnover at pond scale did not translate into large inter-annual faunal changes at regional scale, as evidenced by the fact that all species except *B. bufo* attempted breeding every season. Thus, despite their differences in precipitation, none of those years seems to have been too adverse for the species breeding in the area. These results are in accordance with the stability expected in such a robust pond network, in which the pond spatial arrangement is supposed to favour the long-term conservation of amphibian species. Such arrangement allows individuals to find a suitable pond within their movement range even in unfavourable years when many ponds do not flood (Fortuna *et al.*, 2006). These results also

show that temporal turnover patterns are scale-dependent and hence the scale of observation is critical when comparing results from different areas. For example, in studies conducted in different Mediterranean temporary pond systems, both Richter-Boix *et al.* (2006) and Jakob *et al.* (2003) found inter-annual changes in the species composition of the larval assemblage (pond scale). However, Jakob *et al.* (2003) reported inter-annual variability at the regional scale (i.e. some species disappeared from the study area in particular years) while Richter-Boix *et al.* (2006) found that all amphibian species regularly bred every year.

High temporal turnover is expected in non-stable habitats (Moreno and Halffter, 2001), such as Mediterranean temporary ponds. Environmental fluctuations provide opportunities for temporal niche partitioning: habitat conditions of a pond will favour different species at different times, depending on their niche requirements (Chesson and Huntly, 1997). Doñana National Park may be a good example of such dynamic systems, where species persist because each is periodically favoured and exhibits boom years when large numbers of metamorphs are produced, rather than constant low-level reproductive success each year (Semlitsch, 2002). From a practical perspective, inter-annual turnover at pond scale provides evidence for the inadequacy of surveys conducted only in one breeding season to characterize the species assemblage associated with a given pond. The relevance of the duration of surveys was stressed by Skelly *et al.* (2003) in the context of decline studies when comparing present-day with historical data. He noticed that a 5-year resurvey would yield negligible changes in population whereas a shorter resurvey (1 or 2 years) would falsely suggest a population decline. In this

Table 2. Results from the repeated measures ANOVA analyses comparing annual richness values (S_{ann}) with cumulative richness values (S_{cum}) and annual richness values (S_{ann}) with rarefied richness values (S_r). Analyses were computed for each breeding season. *F* statistics, degrees of freedom and *P*-values are shown

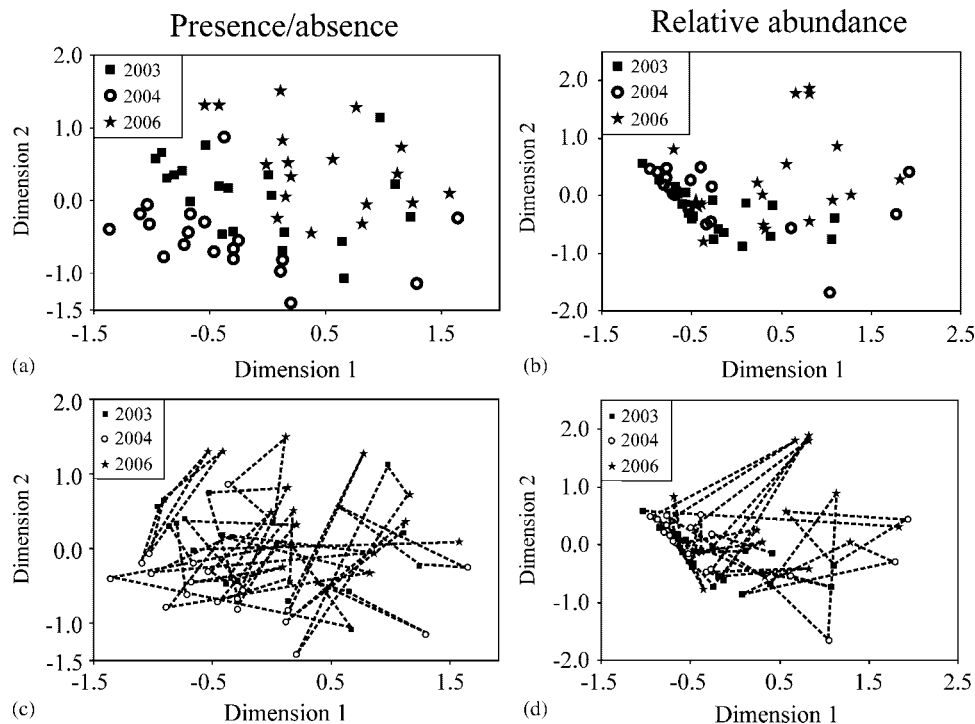| Year	Annual vs Cumulative	Annual vs Rarefied
2003	$F_{1,20} = 26.667$; $P < 0.001$	$F_{1,20} = 7.806$; $P = 0.011$
2004	$F_{1,18} = 53.581$; $P < 0.001$	$F_{1,18} = 31.412$; $P < 0.001$
2006	$F_{1,18} = 33.996$; $P < 0.001$	$F_{1,18} = 0.030$; $P = 0.864$

Figure 4. Ordination plot showing the dissimilarity in assemblage composition among ponds and years based on presence absence-data (NMDS stress = 0.252) (a) and (c) and relative abundance data (NMDS stress = 0.159) (b) and (d). Each case represents the species assemblage composition of a given pond in a particular year (c) is the same ordination as (a) but cases corresponding to the same pond have been joined with discontinuous lines to show the temporal turnover at pond scale (i.e. cases corresponding to the same pond would appear aggregated under low temporal turnover). The same applies to (b) and (d).

study, we also noted that environmental fluctuations probably affected all the pond assemblages in a similar way. So, in any given year, the pond assemblages tended to be similar although not unequivocally distinguishable from assemblages of different years. This result reinforces the relevance of year-specific environmental conditions (i.e. hydrological or meteorological) in the species composition of the larval assemblage which Jakob et al. (2003) pointed out for a different Mediterranean temporary pond system.

In the study ponds, analyses at species level (proportion of occupied ponds and overall species abundance) were useful in indentifying which species showed the most remarkable inter-annual changes. However, they lacked statistical power and therefore had to be complemented with analyses focused on the assemblage level, such as species richness comparisons or the assemblage-composition-based approach. In general, large fluctuations in species occupancy were observed, probably caused by differences in rainfall timing and quantity, which conditioned the availability and characteristics of breeding sites. The breeding success of urodele species decreased in 2006, a year of scarce autumn rainfall, which agrees with previous studies reporting that Triturus species were negatively affected by delayed rainfall, both in the study area (Díaz-Paniagua, 1998) and in a different Mediterranean temporary pond system (Jakob et al., 2003). This result shows that, while occasional dry seasons may not compromise the conservation of urodele species in the study area, a long period of scarce rainfall may severely affect their populations. Low occurrence and abundance of urodeles might have favoured the higher occupancy and overall relative abundance of species such as B. calamita or D. galganoi, which would have faced lower predation pressure, as pointed out by Jakob et al. (2003) in a similar study. These species, which mostly breed in ephemeral unpredictable habitats (Díaz-Paniagua, 1990), probably benefited from the reduction in hydroperiod in 2006 as well. Hydroperiod constraints would also explain the marked decrease in the proportion of ponds these two species occupied in an extremely wet year (2004), when duration of flooding increased in ponds. This reduction in occupancy and overall relative abundance, also noticeable in most other species, could also be due to the formation of additional ponds in the surrounding area, which were not included in this study but may have provided alternative breeding sites for amphibian species.

Patterns of temporal turnover in amphibian communities have previously been documented in the literature (Trenham et al., 2003; Werner et al., 2007) and have been explained by various mechanisms, such as extinction/colonization processes, no breeding activity and movements among ponds (Werner et al., 2007). Although further research is required to understand the processes underlying inter-annual turnover in the study area, two of these mechanisms, movements among ponds and no breeding activity, seem more feasible. We think that the high connectivity in the system of temporary ponds (Fortuna et al., 2006) probably favoured movements of individuals from one pond to another. Inter-pond movements in response to changes in habitat suitability have been reported in amphibian populations, which perceived a cluster of ponds, rather than a single one, as the same breeding unit (Petranka et al., 2004). On the other hand, the observed decrease in the occupancy of some species may support the hypothesis of individuals avoiding breeding in particular ponds and seasons, although not in the entire study area. In any case, none of these events (inter-pond movements and occasional lack of breeding activity) would necessarily affect population size at regional scale and, therefore, none of them should be considered detrimental for the preservation of amphibian species in the area.

From a methodological perspective, two alternative approaches are provided to evaluate temporal turnover at the assemblage level depending on the type of data available. In the richness-based approach, we propose comparisons between annual and cumulative richness, as recommended by Romanuk and Kolasa (2001), complemented with comparisons between annual and rarefied richness, to discard potential biases caused by simple accumulation of sampling effort (Gotelli and Colwell, 2001). As a case in point, in the present study, the absence of significant differences between annual richness in 2006 and rarefied richness for an equivalent sampling effort revealed that, in 2006, differences between annual richness and cumulative richness were not unequivocally attributable to species turnover. On the other hand, the assemblage-composition-based approach evaluates temporal turnover from species presence–absence or relative abundance data. As a major advantage, it quantifies temporal turnover in an easy-to-compute single value for the entire study period and study area, which may enable comparisons with different areas or periods of time. A similar approach, also based on dissimilarity indices, was previously applied to quantify temporal turnover in an amphibian metacommunity (Werner et al., 2007). Although temporal turnover can be computed from any community dissimilarity index (i.e. Bray–Curtis, Jaccard), we recommend the use of indices that yield similarity values independent of richness variation, such as Morisita–Horn or Simpson. These indices will identify the replacement of species in the assemblage (substitution of some species by others) as inter-annual turnover but not the loss of species from the assemblage (reduction in species richness). Thus, they provide complementary information to richness-based approaches. Moreover, patterns of temporal turnover measured with abundance data also complement those obtained with presence–absence data. The identity of species breeding in a pond may not change from year to year while its abundance does. So, it can be interpreted that although all species breed every year, breeding success largely varies from year to year and a given year is not equally favourable for all species. As a result, this alternation in breeding success contributes to the medium-term preservation of all species in the assemblage.

This study contributes to the knowledge of the effects of temporal scale in biodiversity assessments. The observed inter-annual turnover in the area suggests that a given pond offers different breeding opportunities over time, being suitable for different species depending on the year. In fact, sites supporting low annual richness are likely to have high species turnover and thus could be important to a larger number of species over a longer time span (White et al., 2006). Thus, from a conservation perspective, the value of a given site can only be assessed when taking into account the temporal dynamism of the pond assemblage. In fact, such temporal dynamism is the most important feature benefiting the conservation of biodiversity in the medium-term. For that reason, it is important to preserve the natural dynamism and spatial variability of temporary pond systems, which will favour the conservation of populations through their intrinsic variability. From an applied perspective, the major implication of this study is that amphibian monitoring should take temporal dynamics of amphibian communities into account

(i.e. conducted during more than one breeding season) to assess the complete diversity associated with a pond.

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