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# Susceptibility to acidification of groundwater-dependent wetlands affected by water level declines, and potential risk to an early-breeding amphibian species

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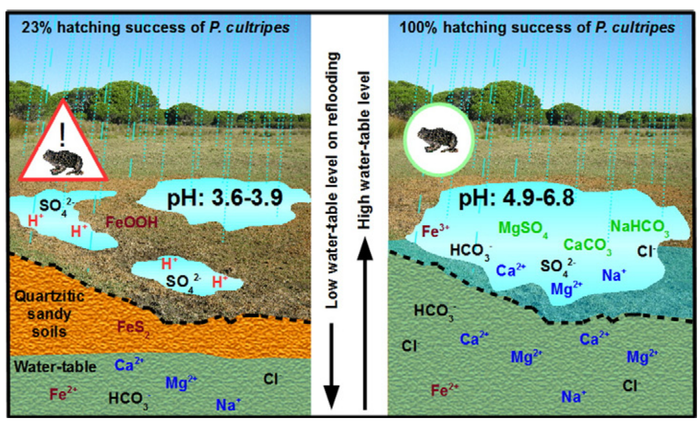
## HIGHLIGHTS

- Both chemical and hydrological factors contributed to inorganic soil acidification.
- Acidification occurred on oxidation of sulfide minerals on re-wetting after drought.
- Early amphibian breeders had to cope with acidification in some sites.
- Water-table declining levels can decrease the hydroperiod length of breeding sites.
- Loss of $Mg^{2+}$ might be an early warning of an eventual water-table decalcification.
- The S/Ca + Mg ratio of the dry sediment was a good predictor of the pH after rainfall.

## GRAPHICAL ABSTRACT

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## ABSTRACT

Eggs of the Western spadefoot toad (*Pelobates cultripes*) reached a 100% mortality in all 29 clutches deposited at a pH below 5.0 in a temporary pond of the Doñana National Park (SW Spain) throughout the wet season of 2006–2007. A similar trend was detected in a neighbouring pond. The proximity of these two ponds to a groundwater pumping area (<1.5 km), prompted us to elucidate the possible links between the reduction in pond hydroperiod over past decades (1989–2008) and the decrease of groundwater pH-buffering capacity. The average hydroperiod had decreased by 4 months since 1998–99 in the pond where the extensive egg mortality had occurred. The total alkalinity, and the $Mg^{2+}$ concentration had also significantly declined in the shallow water-table since 1998–99, from an average of 8.56 to 0.32 meq $l^{-1}$, and of 3.57 to 1.15 meq $l^{-1}$, respectively. This decline of the shallow groundwater buffering capacity could turn this pond more susceptible to the inorganic acidity associated with pyrite oxidation as the sediment S content was often above 0.03%. The initial ratio of S/Ca + Mg in the

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Water-table drawdown
Temporary ponds

summer dry sediment was a good predictor of pore-water pH on re-wetting after desiccation ($r^2 = 0.802$, $p < 0.01$). Therefore, this ratio can give some anticipation to mitigate the impact of acidity on toad hatching before these temporary ponds are reflooded on the next wet season. Our results suggest that the long-term damage to pond water levels can trigger a potential risk of soil acidification in the presence of iron-sulphide minerals.


## 1. Introduction

Since groundwater acidification was defined as the alteration of the geochemical equilibrium of soils that results in a decrease of the pH-buffering capacity of groundwater (de Caritat, 1995; Hansen and Postma, 1995), it has become an ever-growing threat to the quality of groundwater in poorly buffered sandy soils (Kjøller et al., 2004; Fältmarsch et al., 2008; Takem et al., 2015). The reason is that quartz-sandy unconfined aquifers provide less base cations ($Ca^{2+}$ and $Mg^{2+}$) to buffer the soil pH than aquifers containing carbonate minerals (Hansen and Postma, 1995). This poorly-buffered aquifers can be exposed to inorganic acidification processes when acid sulphate soils are present. Contrary to the atmospheric deposition of acid rain over large areas of the Earth's surface following wind currents (Likens et al., 2000), acidification in sulphate acid soils can be site-specific and more easily overlooked because it is largely controlled by soil inherent properties, S abundance and water drainage (Fältmarsch et al., 2008; DER, 2015). Acidification will then occur when the buffering capacity of the soil/sediment is insufficient to compensate for the acids produced during iron-sulphide oxidation as it has been shown in both natural acidic sulfate environments (Basset et al., 1992) and acid-mine drainage lakes (Neumann et al., 2013). Additionally, the combination of desiccation and sulphate polluted groundwater has also the potential to cause a significant pH decline in freshwater peatlands and wet meadows (Lamers et al., 1998). Artificially-lowered groundwater tables can also trigger acidification processes as a result of iron-sulphide oxidation (Appleyard and Cook, 2009).

The adverse effects of acidification on the aquatic biota are well established, particularly on fish populations and invertebrate communities (Driscoll et al., 1994; Fältmarsch et al., 2008; McCullough and Horwitz, 2010). In contrast, the influence of acidification on amphibians remains a contested issue. Despite a lethal pH for amphibian embryos has been established in many species under laboratory conditions (Pierce, 1985; Freda, 1986; Freda et al., 1991; Wells, 2007), the negative effects of acidity have proved more difficult to quantify in field populations. Additionally, amphibians are vulnerable to a multitude of other stressors, such as the spread of pathogens (Blaustein et al., 1994; Carey et al., 1999; Daszak et al., 2003; Woodhams et al., 2008), UV-B radiation (Häkkinen et al., 2001), pesticides (Baker et al., 2013), and exotic predators (Adams, 2000; Cruz et al., 2008). Therefore, acidification alone has not been considered a major cause of widespread amphibian decline because various likely causes can contribute to both large- and fine-scale declines of amphibians (Beebee and Griffiths, 2005; Räsänen and Green, 2009; Wells, 2007). Soil acidification, however, can trigger the mobilization of aluminium (and trace metals) into soil water which can indirectly impact amphibian populations. In this sense, those streams receiving the discharges of acid-mine drainage from abandoned coal mining activities have been reported to exhibit significantly lower salamander abundances than reference sites in the Appalachian mountains (Schorr et al., 2013). Notwithstanding the long chain of cascading effects that acid/metal pollution can exert on an ecosystem, amphibians are still regarded as sentinels of general environmental degradation because of their finely-tuned responses to chemical changes in their habitat. In this sense, Eggert et al. (2006) suggested that the narrow habitat requirement of Spadefoot toads might have linked their survival to the poorly-buffered waters of sandy aquifers which, in turn, are habitats that have suffered complex environmental changes in the last 50 years. Spadefoot toads have a preference for aquatic systems on sandy areas in which they burrow during daytime and, as stated before, quartz-sandy unconfined aquifers are particularly susceptible to acidification. The Western spadefoot toad, *Pelobates cultripes*, is presently classified as NT (near threatened) due to its decreasing population trend affected by different causes, such as the introduction of exotic species, agricultural intensification, tourism, or wetland drainage (IUCN, 2013). This species distribution is largely restricted to the Iberian Peninsula and a few locations in S France though several populations have become extinct in NW Spain in the past three decades (Crottini et al., 2010).

A better understanding of complex environmental changes in the breeding habitats of *P. cultripes* could be achieved in those groundwater-dependent wetlands which have been the focus of long-term monitoring studies, such as the temporary ponds of the Doñana National Park (SW Spain), one of the most important wetland areas in Europe. The shallow water-table of the Doñana unconfined aquifer can feed over 3000 small temporary ponds after heavy rains and thus, provide excellent breeding sites for 8 out of the 13 amphibian species found in SW Spain, including *P. cultripes* (Díaz-Paniagua et al., 2010). Although this Park exhibits a very high protection status (Natural World Heritage Site, Biosphere Reserve, Ramsar site, SPA for Birds), it entered the Montreux Record of Ramsar sites under threat in 1990. Numerous publications have warned about a progressive water-table drawdown due to intensive groundwater pumping in many areas since the early 1980s (Hollis et al., 1989; Suso and Llamas, 1993; Trick and Custodio, 2004; Manzano et al., 2007; Díaz-Paniagua and Aragonés, 2015). One of these areas is the tourist village of Matalascañas, where groundwater abstraction for urban water supply has been reported to reduce the hydroperiod and damage the vegetation in those ponds closer to the pumping area (Serrano and Serrano, 1996; Zunzunegui et al., 1998; Muñoz-Reinoso, 2001; Serrano and Zunzunegui, 2008; Gómez-Rodríguez et al., 2010). Sediment from some of these ponds has been classified as Typic Sulfaquent due to a relative high content in S (Siljeström et al., 1994) as siliceous sands often cover shallow iron oxide deposits in iron-rich groundwater seepage areas (Reina et al., 2015). Additionally, these ponds are poorer in soil carbonates than other Doñana wetlands (Florencio et al., 2014). Therefore, this combination of features make these ponds suitable sites to explore the environmental impact of anthropogenic acidification in groundwater-dependent wetlands in sandy aquifers. To our knowledge, this is the first amphibian field study on the environmental effects of acidification caused by the hydrological alteration of natural acid sulphate soils. The large spatio-temporal variability of this kind of impact on aquatic biodiversity has been argued as the main cause of the scarce literature on this issue (Fältmarsch et al., 2008). We have investigated an unprecedented event of egg mortality of *P. cultripes* that took place in one breeding site with very low water pH in 2006–07. These toads are early breeders that lay their first clutches in temporary ponds just immediately after they begin to flood following summer desiccation (Díaz-Paniagua, 1992). Consequently, this species might be particularly vulnerable to acidification. Our working hypothesis is that the reduction in the hydroperiod recorded in some of these ponds has altered the water-table hydrochemistry over the last decades and, in particular, the capacity to buffer water pH when the ponds start to flood after summer desiccation. We have used recent and previous pond records of water-table depth and hydrochemistry, and assumed that more remote ponds to the pumping wells can be used as reference sites. Our objectives are: (1) to report on the extent of an egg mortality event of *P. cultripes* in two breeding sites during field monitoring in 2006–08; (2) to examine whether these ponds have reduced their pH buffering capacity due to changes in the chemical composition of the shallow water-table; (3) to find out the source of site-specific soil acidification on re-wetting; and (4) to use the ratio S/(Ca + Mg) in the dry sediment as a preliminary tool to assess the potential risk of *P. cultripes* breeding sites to soil acidification.

## 2. Material and methods

### 2.1. Study area

The Doñana region (37°N, 6°W) extends along the coastal plain of the Gulf of Cádiz from the estuary of the Tinto River to the left bank of the Guadalquivir River, and inland from the coastline to the northern uplands bordering the Iberian Pyrite Belt, which hosts the massive Rio Tinto sulphide deposits (Van Geen et al., 1997). This region has a Mediterranean climate with Atlantic influence, generally classified as dry subhumid. Rainfall is quite variable, both within a year and over the years, with a 520 mm yearly average, about 80% of which is distributed throughout a wet period from the end of September to the beginning of April. Summers are very dry and hot, while winters are short and mild. Water balance is generally deficient as rainfall exceeds evapotranspiration only during 3–4 months of the year (Siljeström et al., 1994). The aquifer below this region (~3400 $km^2$) borders the Tinto estuary and extends westward to the Guadiamar and Guadalquivir rivers (Fig. 1). The base of the aquifer is comprised of marine blue marls of the Miocene age upon which sands, silts, and silty-clay materials of aeolian, fluvial

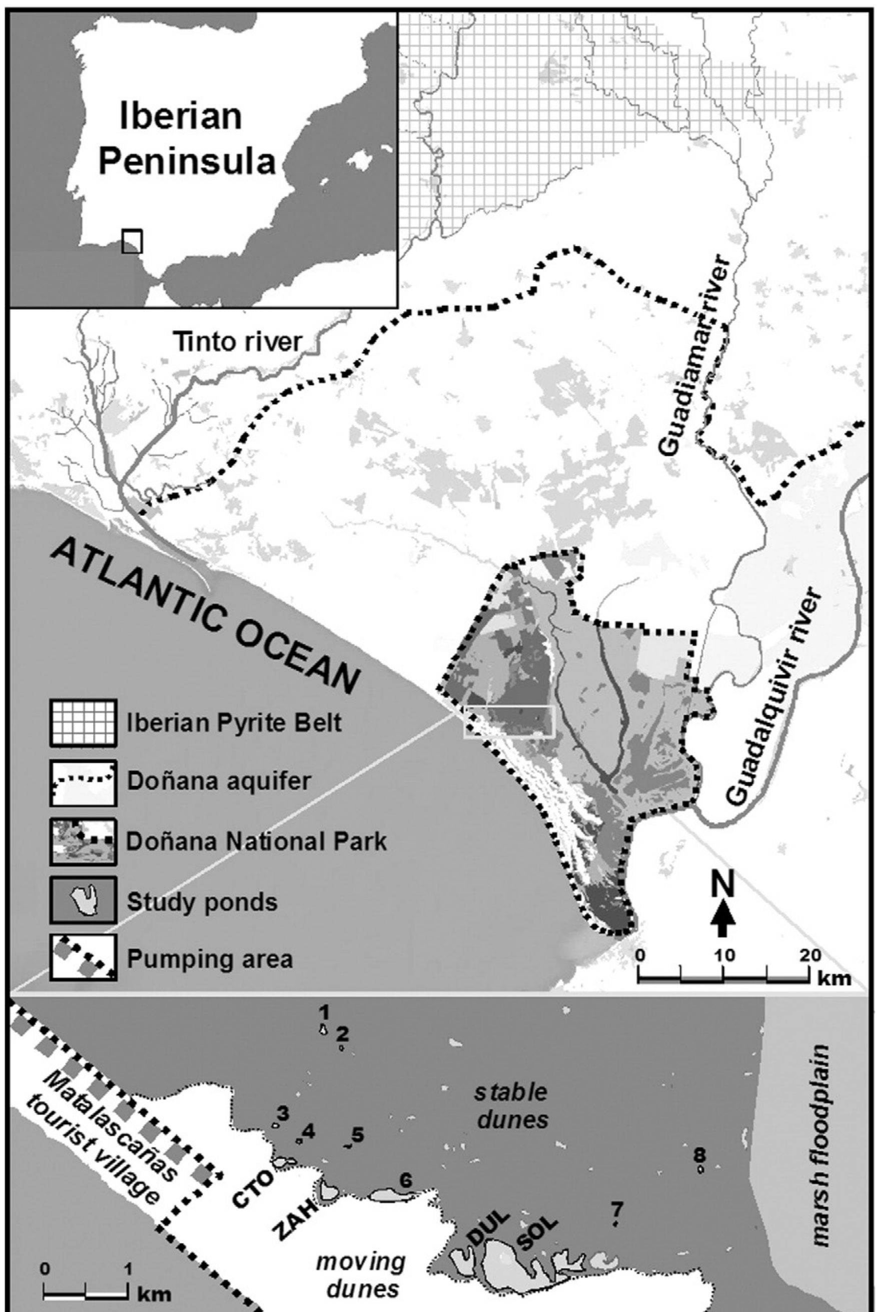


**Fig. 1.** Map of the Doñana region (above) and location of the study ponds (below) in relation to the groundwater pumping area. Surface and water-table chemical analysis were monitored in four ponds (CTO, ZAH, DUL and SOL) while the sediment ratio of S/(Ca + Mg) was measured in all 12 ponds.

and estuarine origins were deposited during the Holocene. The aeolian sands create an unconfined aquifer with a shallow water-table and several flow systems that feed numerous ponds, while the groundwater is confined below a 100-m-thick silty-clay layer in the central marsh floodplain (Llamas, 1990). Thus, the Doñana temporary ponds are fed by freshwater (rainfall, runoff and groundwater discharge), with no other influence from the sea than airborne deposition. They range widely in size (from rain pools to a shallow lake of about 100 ha) and in flooding duration following the general seasonal rainfall pattern of a wet period in autumn-winter to a dry period in spring-summer (Serrano et al., 2006). Some of these wetlands have been considered as flow-through lakes, that is, they can receive water and solutes through seepage from neighbouring ponds located at slightly higher altitudes (Sacks et al., 1992). The study ponds are located in depressions between the stable sands fixed by vegetation and the moving dune front, and at different distances from the Park border that limits the pumping area of Matalascañas (Fig. 1): from ~0.7 km (CTO pond) to >4 km (SOL pond). This pumping area for urban water supply currently includes 10 deep wells (~150 m) placed in 5 double stations lined up along the Park border (Fig. 1). Vegetation around the ponds is dominated by Mediterranean shrubland, with species composition closely related to the water table depth (Zunzunegui et al., 1998). This woody plant community is mainly dominated by *Erica scoparia*, *Calluna vulgaris*, *Ulex minor*, with the occasional presence of *Tamarix canariensis* and isolated individuals of *Quercus suber* left from the original oak-tree woodland. Additionally, *Halimium halimifolium* and *U. australis* can be found in the transition to the xerophityc community of drier areas. The most common wetland species are *Myriophyllum alterniflorum*, *Ranunculus peltatus* and *Eleocharis multicaulis.* Rushes (*Juncus maritimus*, *J. acutus* and *Scirpus holoschoenus*) commonly grow on the pond margins over grassland dominated by *Panicum repens* and *Cynodon dactylon*, which also invade the pond basins during dry periods (Serrano and Zunzunegui, 2008; Fernández-Zamudio et al., 2016). Extensive limnological descriptions of these temporary ponds can be found elsewhere (Serrano and Toja, 1995; Serrano et al., 2006; Gómez-Rodríguez et al., 2009; Florencio, 2010).

### *2.2. Water and sediment chemistry*

The ionic composition of the shallow water-table (<2 m) of four ponds (CTO, ZAH, DUL, and SOL, Fig. 1) was measured in groundwater samples collected from piezometers using ceramic cups (Eijkelkamp, Agrisearch, Giesbeek, The Netherlands) in May 2006. We investigated changes in the ionic composition of the shallow water table by comparing our results and those collected in May, October 1999 and November 2000 (Lozano, 2004) with previous records collected in March, July and September 1991 (López et al., 1994). In May 2006, water-table samples were divided into two parts. One part was fixed with 1% nitric acid and analyzed for elemental composition using an Inductively Coupled Plasma Mass Spectrophotometer (ICP-MS X-series, Thermo, Waltham, MA, USA). The other part of the samples was fixed with 0.125 g $L^{-1}$ citric acid and analyzed for $CO_2$ and $HCO_3^-$ concentrations using a 0525 HR infrared carbon analyzer (Oceanography International).

The potential risk to an early-breeding amphibian (*P. cultripes*) was estimated by calculating the ratio of S/(Ca + Mg) in sediment samples collected in 12 ponds across the study area during the dry season (before breeding took place), and then measuring pore-water pH on artificial re-wetting to simulate early breeding conditions according to Lucassen et al. (2002). Then, sediment was sampled from 2 to 14 sampling stations within each of the 12 study ponds (Fig. 1) in June 2007 by collecting approximately 350 ml of sediment from the upper 20 cm of the pond bottom with a hand corer. The sediments were partly dried (105 °C, 24 h) after which the organic matter content was determined by loss on ignition (550 °C, 4 h). Dried sediment was ground and 200 mg were digested for 17 min in 4 ml 65% $HNO_3$ and 1 ml 30% $H_2O_2$ in an Ethos D Microwave (Milestone, Sorisole, Lobardy, Italy) for the determination of elemental composition (Kingston and Haswell, 1997). The sediment samples were desiccated in the laboratory by gradually air-drying 100 g of fresh sediment in 250 ml polyethylene pots at room temperature for two weeks. Then, demineralized water was added to the original water content and the rewetted sediments were shaken at 100 rpm for 2 h. The pH of the sediment pore-water was measured using a standard Ag/$AgCl_2$ electrode connected to a radiometer Copenhagen type PHM 82 standard pH meter. Pore-water was collected with Teflon rhizons (Eijkelkamp, Agrisearch, Giesbeek, The Netherlands) connected to 30 ml vacuum serum bottles, which was then analyzed for elemental composition following the same procedure described above.

### *2.3. Hydro-meteorological and water pH records of two ponds*

Daily rainfall data were obtained from the meteorological station of Palacio de Doñana (RBD-CSIC). Hydrological data were collected in CTO and ZAH ponds from October 1989 to September 2008, at 1–8-week intervals during 19 hydrological years (1989–2008). Small piezometers (PVC tubes, 4–6 cm diameter, filtered tipped) were placed in each pond basin at 1.5–2.0 m depth to record the depth of the water-table since 1989. Surface water levels were also monitored on the same 1–8 weekly basis.

### *2.4. Egg monitoring*

Field monitoring of amphibian breeding habitats started in the Doñana ponds decades ago (Díaz-Paniagua, 1988, 1990, 1992; Díaz-Paniagua et al., 2005; Gómez-Rodríguez et al., 2009). In the present study, CTO and ZAH ponds were monitored to detect toad spawns and egg mortality during the autumn-winter of 2006–07 and 2007–08. The selection of these two ponds in particular was due to the fact that *in situ* water pH was known to be very low on receiving rainwater after summer desiccation (Serrano and Toja, 1995; Florencio, 2010). Although CTO had not been as intensively surveyed for amphibians as ZAH pond during 2003–06 (Gómez-Rodríguez et al., 2009), previous observations revealed that *P. cultripes* was commonly found in CTO pond in 1998 (Fahd et al., 2007). Although no pond can be considered representative of the whole network of the Doñana temporary ponds, CTO and ZAH ponds are two neighbouring ponds of similar-size, located within the Biological Reserve which is the area that holds the largest amphibian richness within the Park (Díaz-Paniagua et al., 2005). In the present study, we searched for clutches of *P. cultripes* in pools across the entire pond basin and marked them with a stake, recorded water pH, and assessed the percentage of damaged eggs as well as their general appearance. The marked clutches were all monitored once every week following marking during 2006/07. A larger effort to record daily pH changes was employed in 2007/08, when daily monitoring was carried out until we could confirm complete egg/embryo mortality or embryo hatching in successful clutches. We easily detected the clutches affected by acidity in the field because their normally translucent jelly acquired a whitish appearance, being shrunk and tightly bunching the eggs inside. The firm jelly trapped the eggs inside and prevented their hatching, finally causing their death.

### *2.5. Statistical analysis*

Differences in water pH were compared for those clutches with high egg mortality versus those clutches that had not been affected by acidity (mostly surviving eggs) using ANOVA after checking normality and equal variance assumptions. When different clutches were grouped only one pH value was considered for the group. For hydrological and chemical data, differences were compared using a Student *t*-test when the data met the requirements, and with a non-parametric Mann-Whitney test when they did not. Spearman's rank correlation coefficients (Rho) were calculated between rainfall and water levels, as well as between pH and sediment ratios. Non-linear exponential decay regression and stepwise forward multiple regression were also performed using SigmaPlot for Windows version 11.0 (Systat Software, Inc.) for the sediment chemistry analyses.

## 3. Results

### 3.1. Field survey of egg mortality of the Western spadefoot toad

During the first heavy autumn rains in November 2006, only a small pool (ca. 3.5$m^2$) was flooded within the CTO pond basin. This pool registered a water pH of 4.1, and four clutches were laid which reached 100% egg mortality (Table 1). A second event of egg laying was detected in December 2006 with only one clutch, which also reached 100% egg mortality at a water pH of 4.5. The third event of heavy rains occurred in February 2007, when the surface pond water had expanded to about 600 $m^2$, and 24 egg clutches were recorded at a mean water pH of 4.3 and, again, mortality reached 100% in all egg clutches (Table 1). No eggs of *P. cultripes* were found in CTO pond during the following rainy season of 2007–08.

In ZAH pond, we monitored two events of toad oviposition during 2006–07 when water pH exhibited a large variation in different pools across the pond basin (Table 1). Then, surviving eggs were those deposited at a water pH ≥ 5.7 (Table 1). Three events of oviposition were monitored during the following breeding season of 2007–08 in ZAH pond. Water pH varied across this pond basin during the first two events, while it remained close to or above 5 in all the sites sampled during the third event (Table 1). Forty-one clutches were monitored during the first oviposition event (November 2007) and 22% of them were affected by acidification. They were located in sites with an average water pH of 4.7, while non-affected clutches were found at an average water pH of 6.5. In the second oviposition event (January 2008), 20 out of 26 clutches were affected, and they were located in water with an average pH of 4.9, while non-affected clutches were found at an average water pH of 6.2 (Table 1). By February 2008 (third oviposition event), all clutches survived despite water pH was occasionally as low as 4.9. In the total survey within ZAH pond, the number of egg clutches affected by mortality had been deposited at a significantly lower pH than the surviving egg clutches: $F(1,70) = 86.742$, $p < 0.01$.

### 3.2. Water-level and hydrochemical records

Both the surface water level and the depth of the water-table below the ponds generally fluctuated following the seasonality of rainfall (Fig. 2). In contrast to ZAH pond, CTO pond was dry during 1999–00, 2001–02, 2005–06, 2007–08, and even in 2006–07 despite the yearly rainfall (755.5 mm) was well above the average of 568 mm calculated over the study period. Consequently, the depth of the water-table in CTO pond did not follow the rainfall as consistently as it did in the case of ZAH pond (Fig. 2). The hydrological cycle of 1998–99 was one of the driest cycles ever recorded, with 220 mm of yearly rainfall. Before this cycle, the yearly average of water-table depth was significantly correlated to the yearly amount of rainfall in both ZAH and CTO ponds (Rho = 0.883 and Rho = 0.800, $p < 0.01$, respectively). After 1998–99, however, the water-table depth in CTO pond was no longer correlated to the yearly rainfall ($p > 0.05$), while a weak correlation remained in ZAH pond (Rho = 0.663, $p < 0.05$). Similarly, the duration of the wet phase during each cycle (or hydroperiod) in the set of 9 years after 1998–99 was, on average, 7.4 and 1.6 months per year in ZAH and CTO ponds, respectively, and this difference was significant (Paired-*t* test, $p < 0.01$). No difference was found between the hydroperiod of both ponds in the 9 years before 1998–99 ($p > 0.05$) which corresponded to an average of 6.7 and 5.8 months per year for ZAH and CTO ponds, respectively. Therefore, the average hydroperiod shortened by four months in CTO pond, while ZAH pond exhibited a reduction of less than one month.

In spite that the sampling frequency was not consistent throughout the 19-year record, the yearly minimum water pH was generally detected at the onset of each rainy season, being as low as 3.3 in CTO pond and 5.0 in the ZAH pond (Fig. 2). Water pH in pools across the pond basins during the filling period was occasionally lower, but for a meaningful comparison, the water pH record was built with field measurements routinely performed in the deepest area within each pond basin. Later in the year, water pH tended to increase in both ponds reaching up to 10.1 in the ZAH pond (Fig. 2). This ability to buffer low pH values was sometimes a very sudden process, such as when the pH increased from 4.0 to 6.8 in just 11 days of heavy rainfall that accumulated 230 mm during November 1989 in CTO pond (Fig. 2).

The hydrochemistry of the shallow water-table changed over time in the four study ponds (Table 2): two ponds with low water pH (CTO and ZAH ponds) and two other ponds with no signs of acidification (SOL and DUL reference ponds). Total alkalinity and the sum of base cations (SBC), particularly $Mg^{2+}$ and $Na^+$, was significantly lower in the set of samples collected during 1991 than in the rest of samples collected during 1999–00 and May 2006, only in the case of CTO pond (Student *t*-test, $p < 0.05$). No changes in the $Ca^{2+}$, $SO_4^{2-}$ or $Cl^-$ concentrations were detected in the water-table of any study pond over time.

### 3.3. Source of soil acidification on re-wetting

The sediment S content of all samples ranged widely (0.01–0.28%) and it was highest in CTO pond (0.03–0.28%). These sediment samples also exhibited the lowest range in pore-water pH (3.3–4.7) compared to the rest of the ponds on artificial re-wetting after desiccation, while ZAH pond displayed the widest range (3.5–7.7). The sediment molar ratio of S/Ca + Mg before re-wetting (or initial S/Ca + Mg) was negatively correlated to the pH of the rewetted sediment pore-water (Rho = −0.880, $p < 0.01$). This ratio was a good predictor of pH on re-wetting as the adjusted $r^2$ was 0.802 ($p < 0.01$) for a non-linear exponential decay regression (Fig. 3). Furthermore, the resulting pore-water pH on re-wetting after desiccation was significantly predicted by this initial ratio ($p < 0.01$) and not by the linear combination of other independent variables in the dry summer sediment, such as the sediment organic matter ($p > 0.05$) or the sediment S content ($p > 0.05$) according to a stepwise forward multiple regression.

Except for Al, which did not show any significant difference ($p > 0.05$), the concentrations of other trace metals in the pore-water were significantly higher ($p < 0.01$) at pH < 5.0 on re-wetting after desiccation in all the study sites according to a Mann-Whitney test (Table 3). At pH < 5.0, the sediment pore-water of the CTO pond registered the highest concentration of Mn, Zn, Sr and Pb (51.6, 15.1, 4.1 and 0.3 mg $l^{-1}$, respectively), while the concentrations of Co and Ni were highest in ZAH pond (1.0 and 0.8 mg $l^{-1}$, respectively).

**Table 1**
The clutch mortality refers to the percentage of monitored clutches of *Pelobates cultripes* in which egg mortality was detected in CTO and ZAH ponds. The water pH was recorded at each site where clutches contained either surviving or dead eggs (a minimum-maximum range is indicated when more than one pH value was recorded). The total number of monitored clutches is also indicated. No toad eggs were detected in CTO pond during 2007–08.

| Pond | Date | Clutch mortality (%) | Water pH dead eggs | Water pH surviving eggs | No. clutches recorded |
|---|---|---|---|---|---|
| CTO | 10/11/2006 | 100 | 4.0 | – | 4 |
| CTO | 05/12/2006 | 100 | 4.5 | – | 1 |
| CTO | 06/02/2007 | 100 | 3.6–4.8 | – | 24 |
| ZAH | 26/10/2006 | 66.7 | 4.5 | 5.7 | 3 |
| ZAH | 06/02/2007 | 72.7 | 3.7–3.9 | 6.9 | 11 |
| ZAH | 22/11/2007 | 22 | 3.9–4.9 | 5.3–6.7 | 41 |
| ZAH | 28/12/2007 | 76.9 | 4.1–5.5 | 5.6–6.7 | 26 |
| ZAH | 17/02/2008 | 0 | – | 4.9–6.8 | 12 |

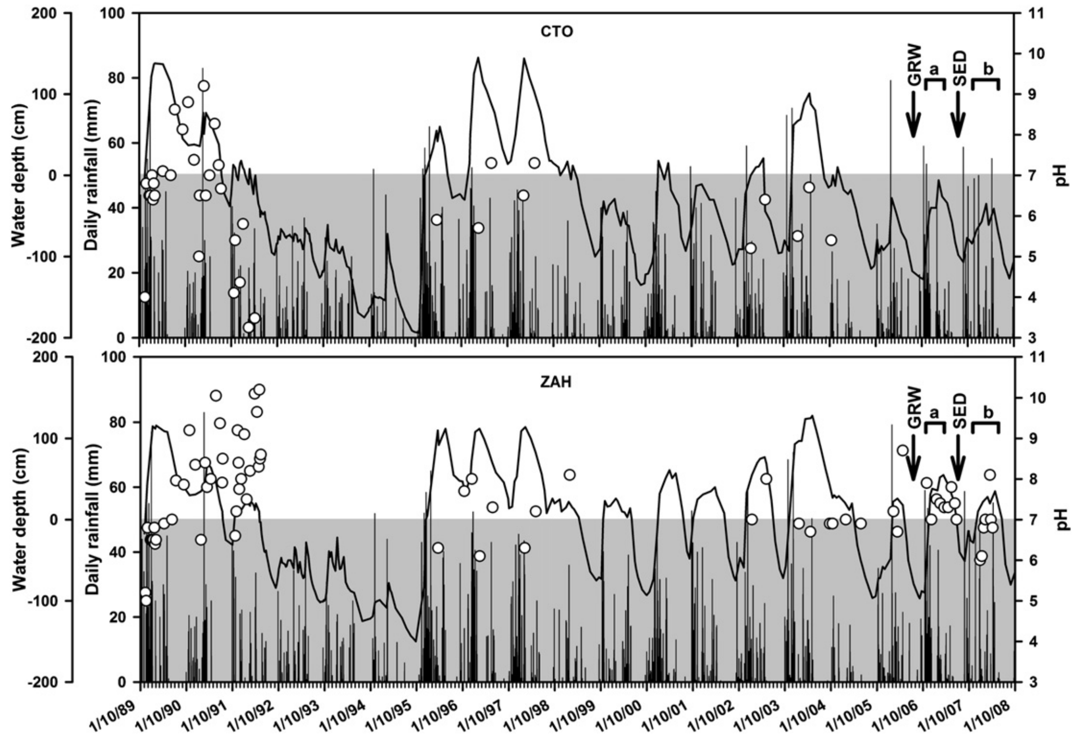


**Fig. 2.** Records of surface water pH (white circles) and water depth (solid line) in CTO and ZAH ponds from 1989–90 to 2007–08. Water depth within the grey area corresponds to the water-table below ground. Vertical bars indicate daily rainfall. The vertical arrows indicate the sampling dates for groundwater (GRW) and sediment (SED) chemical analysis in May 2006 and June 2007, respectively; *a* and *b* indicate field surveys for amphibian eggs (2006–07 and 2007–08, respectively).

## 4. Discussion

The wide range of S abundance in the sediment of the study ponds was similar to that recently reported by Kohfahl et al. (2016) in the soil profile of samples collected from the southern area of the Doñana National Park (0.01–0.17%). Besides this highly variable distribution of S, some other features may indicate the presence of acid sulphate soils in the Doñana temporary ponds according to DER (2015): these ponds are located in coastal sand dunes of recent geological age (Holocene) where the soil pH is neutral or slightly acidic, and ochre deposits are commonly found due to high concentrations of hydroferric oxides in seepage areas where iron-rich floating films are present (Reina et al., 2015). Therefore, the most likely source of inorganic acidification at the onset of the rainy season was the excess of $H^+$ formed during the oxidation of sulphide minerals. Given that the complete oxidation of sulphide minerals was not measured, the water pH monitored during the early wet season was likely an underestimation of the potential acidity encountered by the toad eggs during hatching. Nevertheless, the toad hatching success can be expected to depend on both the severity and duration of the exposure of eggs to acidic conditions in a similar way as reported for fish populations in rivers and estuaries receiving the drainage of acid sulphate soils (Fältmarsch et al., 2008). Then, a brief exposure could explain the survival of all eggs during the third oviposition event in February 2008 despite water pH was occasionally as low as 4.9. Also, the toxicity of $SO_4^{2-}$ to the aquatic biota has been reported to decrease with increasing water hardness though the exact effect of this influence on the membrane permeability has not yet been established (Elphick et al., 2011). Therefore, we have provided only a preliminary assessment of the potential risk of acidification to early amphibian breeders by means of the sediment ratio of S/(Ca + Mg). In agreement with the findings by Lucassen et al. (2002) in several Dutch non-coastal minerotrophic wetlands, our results indicated that this ratio could

**Table 2**
Mean concentrations of the sum of base cations (SBC), total alkalinity, and $SO_4^{2-}$ (meq $l^{-1}$) in the shallow water-table of those ponds with signs of recent acidification (CTO and ZAH ponds) and without them (SOL and DUL ponds) at different dates. *Significant differences (Student *t*-test, $p < 0.05$) between all samples collected during 1991 and the rest of dates (1999–2000 and May 2006).

| | | CTO | ZAH | SOL | DUL |
|---|---|---|---|---|---|
| 1991[a] | SBC | 22.85* | 6.00 | 8.23 | 10.18 |
| 1991[a] | Alkalinity | 8.56* | 7.21 | 11.76 | 3.49 |
| 1991[a] | $SO_4^{2-}$ | 3.19 | 2.34 | 2.44 | 1.65 |
| 1999–2000[b] | SBC | 4.94* | 5.76 | 2.08 | 1.78 |
| 1999–2000[b] | Alkalinity | 0.20* | 0.98 | 0.56 | 0.57 |
| 1999–2000[b] | $SO_4^{2-}$ | 3.00 | 0.67 | 0.13 | 0.06 |
| May 2006[c] | SBC | 5.38* | 8.63 | 3.38 | 6.27 |
| May 2006[c] | Alkalinity | 0.68* | 8.20 | 1.75 | 1.68 |
| May 2006[c] | $SO_4^{2-}$ | 3.25 | 4.52 | 0.33 | 1.12 |

[a] López et al. (1994).
[b] Lozano (2004).
[c] Present study.

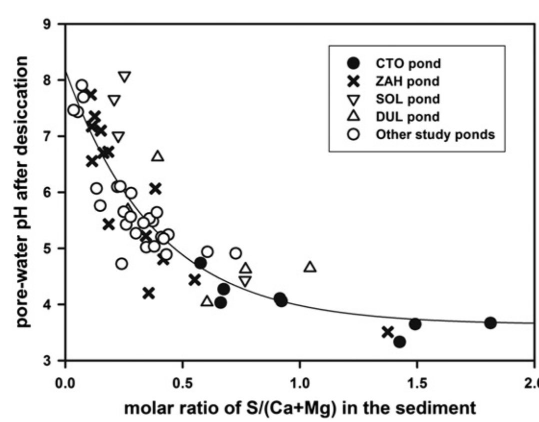


**Fig. 3.** Pore-water pH in artificially rewetted sediment after desiccation in relation to the initial molar ratio of S/(Ca + Mg) in the sediment of the study ponds in June 2007. Exponential decay regression as solid line.

**Table 3**
Median concentrations (mg $l^{-1}$) of trace metals in the pore-water of sediments on re-wetting after desiccation, and their significant difference (**$p < 0.01$) between sampling stations with a pore-water pH < 5.0 and pH > 5.0 in all study ponds according to a Mann-Whitney test.

| | Mn | Zn | Sr | Ni | Al | Fe | Cu | Co | Pb |
|---|---|---|---|---|---|---|---|---|---|
| pH < 5.0 | 4.48** | 1.03** | 1.08** | 0.28** | 0.10 | 0.15** | 0.08** | 0.063** | 0.021** |
| pH > 5.0 | 0.22** | 0.28** | 0.35** | 0.13** | 0.07 | 0.06** | 0.04** | 0.002** | 0.004** |

significantly predict the pH of the sediment pore-water on re-wetting. Although a full assessment of the presence of acid sulphate soils is recommended to develop action plans (DER, 2015), this ratio performed on the dry sediment could provide some anticipation to mitigate the impact of acidity on toad hatching before these temporary ponds are reflooded on the next wet season.

Solute mass balance studies have shown that alkalinity and base cations are depleted in the surface water of these ponds relative to groundwater due to evaporative concentration, but they can be replenished as soon as the water-table rises above the surface after heavy rains (Sacks et al., 1992; Serrano and Toja, 1995). Therefore, those sites with a high ratio of S/(Ca + Mg) most likely relied on the groundwater discharge for a supply of base cations to neutralise the soil acidity of rainpools across the pond basin at the onset of the rainy season, such as in the case of ZAH pond where all toad eggs survived at pH 4.9–6.8 in 2008. In wetlands of the Murray-Darling Basin (Australia), sediments with a pH ≥ 6.5 tended to have high acid-neutralizing capacity that could prevent acidification even after sulphide oxidation (Baldwin et al., 2007). In the present work, the shallow water-table remained below the surface of CTO pond during 2006–08 and thus could not contribute to buffer the surface water pH. This long-term damage to the groundwater discharge could eventually turn these ponds more susceptible to inorganic acidification as we have detected a significant reduction in the concentrations of alkalinity, $Mg^{2+}$ and the sum of base cations in the shallow water-table of CTO pond over the past decades. A previous study in this area also reported similar hydrochemical anomalies in the groundwater below CTO pond, with a low pH (<6.0), and a high $SO_4^{2-}$ concentration that rendered this water of Na-$SO_4$-Cl type while water type was dominantly Na-Cl in ZAH, DUL and SOL ponds (Lozano, 2004). There are two hydrological alterations that contribute to explain the loss of base cations in the shallow-water table. One is the observed reduction in CTO pond hydroperiod compared to the rest of the study ponds since 1998–99, and the other is a decline in the flow of deep groundwater discharge which has already been reported for the nearby Brezo pond due to groundwater abstraction for urban water supply (Muñoz-Reinoso, 2001). In the first mechanism, the shorter the duration of flooding, the shorter the time for subsurface sorption on soil particles, and the more cations that migrate into deeper soil layers because solutes will move downwards following the regional aquifer flow path (Sacks et al., 1992). In the second one, the lesser the upflow of groundwater volume discharge, the more likely that cations will not be recovered from the unsaturated zone to the surface. The outcome of both mechanisms is the depletion of cations in the pond surface water. Likewise, two decades of unsustainable groundwater exploitation have caused the leaching of base cations from soils within cones of depressions of pumping wells in a sandy unconfined aquifer of Western Australia (Appleyard and Cook, 2009). Given that $Ca^{2+}$ is retained in the soil to a greater extent than $Mg^{2+}$ in Quaternary sandy coastal aquifers (Hansen and Postma, 1995), the observed significant reduction of $Mg^{2+}$, but not of $Ca^{2+}$, in the shallow water-table below CTO pond should be considered as an early warning of the loss in the acid-neutralizing capacity of this pond. Decalcification in silica-dominated soils is an irreversible process which has already been reported in a German unconfined aquifer due to the combined effect of natural and anthropogenic processes including fluctuating water-tables, oxidation processes, agricultural exploitation, past acid rain deposition, and increased groundwater withdrawal (Wiegand, 2009).

The presence of trace metals in the sediment pore-water of samples with a pH < 5.0 can be of concern because elevated concentrations of heavy-metals have been shown to be a major source of toxicity to amphibians (Elphick et al., 2011). The study ponds are a good example of softwater with plenty of sulphide-minerals and trace metals. The widespread occurrence of trace metals in the Doñana National Park has long been considered indicative of chronic pollution due to the intensive mining activity of the Iberian Pyrite Belt (Albaigés et al., 1987). This view was later supported by geochemical analysis of sediment cores collected from the continental shelf in the Gulf of Cádiz (Van Geen et al., 1997), and the Guadiamar River catchment pre-dating the 1998 Aznalcóllar tailings dam failure (Turner et al., 2008). More recently, the presence of elevated concentrations of heavy metals (including As) in sediment samples of the Doñana National Park has also been attributed to their geogenic origin from ore minerals of the Iberian Pyrite Belt (Kohfahl et al., 2016).

Thirty years ago, the Western spadefoot toad was the most abundant species in the Doñana National Park (Valverde, 1967; Díaz-Paniagua et al., 2005). Predation by the red swamp crayfish has been considered as a likely factor contributing to a recent population decline, particularly in the Doñana marshland (Díaz-Paniagua et al., 2005). While the occasional mortality caused by acidification will probably not drive these populations to extinction, these acidification events are indicative of a general deterioration of their breeding habitats. Reducing the length of the aquatic phase in a temporary pond means reducing the probability of tadpoles to complete metamorphosis in an even more stressed scenario whenever acidification events take place. Nevertheless, the survival of the Doñana amphibian community can be ensured if momentary unfavorable conditions can be overcome by different species finding a suitable habitat across the whole temporary pond network (Gómez-Rodríguez et al., 2009). Our results are in line with other studies showing that the effect of acidification on amphibians is difficult to assess due to the combination of confounding factors acting over a long time. In The Netherlands, a decrease of 25 to 80% in different species since 1950 has been recently reported to be caused by a combination of acidification, eutrophication, pollution with pesticides, and changes in vegetation (Fedorenkova et al., 2012). A probability risk approach ranked pH as the primer threat to anuran populations in a list that included a total of 18 stressors, such as copper, diazinon, ammonium, and endosulfan (Fedorenkova et al., 2012).

## 5. Conclusions

The present results showed that the sediment molar ratio of S/(Ca + Mg) can be a preliminary tool to indicate a potential risk of inorganic acidification to amphibian early breeders using dry sediments. The susceptibility of breeding sites to this potential risk can increase in wetlands with severe hydrological alterations. Long-term monitoring of the hydrology and chemistry of the shallow water-table has, thus, proved essential to assess how complex chemical interactions are being altered by a growing human pressure on water resources. The proposal of a safety zone around wetlands, based on the relationship between the abstraction rate and the wetland distance to the pumping well (Bekesi and Hodges, 2006), might also be a feasible solution if groundwater-dependent ecosystems are to be protected.

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## References

Adams, M.J., 2000. Pond permanence and the effects of exotic vertebrates on anurans. Ecol. Appl. 10, 559–568.

Albaigés, J., Algaba, J., Arambarri, P., Cabrera, F., Baluja, G., Hernández, L.M., Castroviejo, J., 1987. Budget of organic and inorganic pollutants in the Doñana National Park (Spain). Sci. Total Environ. 63, 13–28.

Appleyard, T., Cook, S., 2009. Reassessing the management of groundwater use from sandy aquifers: acidification and base cation depletion exacerbated by drought and groundwater withdrawal on the Gnangara Mound, Western Australia. Hydrogeol. J. 17, 579–588.

Baker, N.J., Bancroft, B.A., Garcia, T.S., 2013. A meta-analysis of the effects of pesticides and fertilizers on survival and growth of amphibians. Sci. Total Environ. 449, 150–156.

Baldwin, D.S., Hall, K.C., Rees, G.N., Richardson, A.J., 2007. Development of a protocol for recognizing sulfidic sediments (potential acid sulfate soils) in freshwater wetlands. Ecol. Manag. Restor. 8, 56–60.

Basset, R.L., Miller, W.R., McHugh, J., Catts, J.G., 1992. Simulation of natural acid sulfate weathering in an alpine watershed. Water Resour. Res. 28, 2197–2209.

Beebee, T.J.C., Griffiths, R.A., 2005. The amphibian decline crisis: a watershed for conservation biology? Biol. Conserv. 125, 271–285.

Bekesi, G., Hodges, S., 2006. The protection of groundwater dependent ecosystems in Otago, New Zeal. Hydrogeol. J. 14, 1696–1701.

Blaustein, A.R., Hokit, D.G., O' Hara, R.K., 1994. Pathogenic fungus contributes to amphibian losses in Pacific Northwest. Biol. Conserv. 67, 251–254.

Carey, C., Cohen, N., Rollins-Smith, L., 1999. Amphibian declines: an immunological perspective. Dev. Comp. Immunol. 23, 459–472.

Crottini, A., Galán, P., Vences, M., 2010. Mitochondrial diversity of Western spadefoot toads, *Pelobates cultripes*, in northwestern Spain. Amphibia-Reptilia 31, 443–448.

Cruz, M.J., Segurado, P., Sousa, M., Rebelo, R., 2008. Collapse of the amphibian community of the Paul do Boquilobo Natural Reserve (central Portugal) after the arrival of the exotic American crayfish *Procambarus clarkii*. Herpetol. J. 18, 197–204.

Daszak, P., Cunningham, A.A., Hyatt, A.D., 2003. Infectious disease and amphibian population declines. Divers. Distrib. 9, 141–150.

de Caritat, P., 1995. Intensifying groundwater acidification at Birkenes, southern Norway. J. Hydrol. 170, 47–62.

Department of Environment Regulation (DER), 2015. Identification and investigation of acid sulfate soils and acidic landscapes. Perth: Acid Sulfate Soils Guideline Series. Department of Environment Regulation, Government of Western Australia Available from https://www.der.wa.gov.au/your-environment/acid-sulfate-soils/69-ass-guidelines.

Díaz-Paniagua, C., 1988. Temporal segregation in larval amphibian communities in temporary ponds at a locality in SW Spain. Amphibia-Reptilia 9, 15–26.

Díaz-Paniagua, C., 1990. Temporary ponds as breeding sites of amphibians at a locality in southwestern Spain. Herpetol. J. 1, 447–453.

Díaz-Paniagua, C., 1992. Variability in timing of larval season in an amphibian community in SW Spain. Ecography 15, 267–272.

Díaz-Paniagua, C., Aragonés, D., 2015. Permanent and temporary ponds in Doñana National Park (SW Spain) are threatened by desiccation. Limnetica 34, 407–424.

Díaz-Paniagua, C., Gómez-Rodríguez, C., Portheault, A., De Vries, W., 2005. Los Anfibios de Doñana. Organismo Autónomo de Parques Nacionales-Ministerio de Medio Ambiente, Madrid.

Díaz-Paniagua, C., Fernández-Zamudio, R., Florencio, M., García-Murillo, P., Gómez-Rodríguez, C., Portheault, A., Serrano, L., Siljeström, P., 2010. Temporary ponds from Doñana National Park: a system of natural habitats for the preservation of aquatic flora and fauna. Limnetica 29, 41–58.

Driscoll, C.T., Likens, G.E., Hedin, L.O., Eaton, J.S., Bormann, F.H., 1994. Change sin the chemistry of surface waters. Environ. Sci. Technol. 23, 137–143.

Eggert, C., Cogălniceanu, D., Veith, M., Dzukic, G., Taberlet, M., 2006. The declining Spadefoot toad, *Pelobates fuscus* (Pelobatidae): paleo and recent environmental changes as a major influence on current population structure and status. Conserv. Genet. 7, 185–195.

Elphick, J.R., Davies, M., Gilron, G., Canaria, E.C., Lo, B., Bailey, H.C., 2011. An aquatic toxicological evaluation of sulfate: the case for considering hardness as a modifying factor in setting water quality guidelines. Environ. Toxicol. Chem. 30, 247–253.

Fahd, K., Florencio, M., Keller, C., Serrano, L., 2007. The effect of the sampling scale on zooplankton community assessment and its implications for the conservation of temporary ponds in south-west Spain. Aquat. Conserv. 17, 175–193.

Fältmarsch, R.M., Åström, M.E., Vuori, K.-M., 2008. Environmental risks of metals mobilised from acid sulphate soils in Finland: a literature review. Boreal Environ. Res. 33, 444–456.

Fedorenkova, A., Vonk, A.J., Lenders, H.J.R., Creemers, R.C.M., Breure, A.M., Hendriks, A.J., 2012. Ranking ecological risks of chemical multiple stressors on amphibians. Environ. Toxicol. Chem. 31, 1416–1421.

Fernández-Zamudio, R., García-Murillo, P., Díaz-Paniagua, C., 2016. Aquatic plant distribution is driven by physical and chemical variables and hydroperiod in a mediterranean temporary pond network. Hydrobiologia 774, 123–135.

Florencio, M. Dinámica espacio temporal de la comunidad de macroinvertebrados de las lagunas temporales de Doñana. PhD thesis. Sevilla: Universidad de Sevilla; 2010.

Florencio, M., Serrano, L., Siljeström, P., Zamudio-Fernández, R., García-Murillo, P., Díaz-Paniagua, C., 2014. The influence of geomorphology on the composition of aquatic flora and fauna within a temporary pond network. Limnetica 33, 327–340.

Freda, J., 1986. The influence of acidic pond water on amphibians: a review. Water Air Soil Pollut. 30, 439–450.

Freda, J., Sadinski, W.J., Dunson, W.A., 1991. Long term monitoring of amphibian populations with respect to the effects of acidic deposition. Water Air Soil Pollut. 55, 445–462.

Gómez-Rodríguez, C., Díaz-Paniagua, C., Serrano, L., Florencio, M., Portheault, A., 2009. Mediterranean temporary ponds as amphibian breeding habitats: the importance of preserving pond networks. Aquat. Ecol. 43, 1179–1191.

Gómez-Rodríguez, C., Díaz-Paniagua, C., Bustamante, J., 2010. Evidence of hydroperiod shortening in a preserved system of temporary ponds. Remote Sens. 2, 1439–1462.

Häkkinen, J., Pasanen, S., Kukkonen, J.V.K., 2001. The effect of solar-UV-B radiation on embryonic mortality and development in three boreal anurans (*Rana temporaria*, *Rana arvalis* and *Bufo bufo*). Chemosphere 44, 441–446.

Hansen, B.K., Postma, D., 1995. Acidification, buffering, and salt effects in the unsaturated zone of a sandy aquifer, Klosterhede, Denmark. Water Resour. Res. 31, 2795–2809.

Hollis, T., Mercer, J., Heurteaux, P., 1989. The implications of groundwater extraction for the longterm future of the Doñana National Park. WWF/UICN/ADENA Mission to Doñana National Park (18–22 November 1988).

IUCN, 2013. The IUCN Red List for Threatened Species. Version 2013.2. International Union for Conservation of Nature, Cambridge Available from http://www.iucnredlist.org.

Kingston, H.M., Haswell, S., 1997. Microwave Enhanced Chemistry. American Chemical Society, Washington DC.

Kjøller, C., Postma, D., Larsen, F., 2004. Groundwater acidification and the mobilization of trace metals in a sandy aquifer. Environ. Sci. Technol. 38, 2829–2835.

Kohfahl, C., Sánchez-Rodas Navarro, D., Mendoza, J.A., Vadillo, I., Giménez-Forcada, E., 2016. Algae metabolism and organic carbon in sediments determining arsenic mobilisation in ground- and surface water. A field study in Doñana National Park, Spain. Sci. Total Environ. 544, 874–882.

Lamers, L.P.M., Van Rozendaal, S.M.E., Roelofs, J.G.M., 1998. Acidification of freshwater wetlands: combined effects of non-airborne sulfur pollution and desiccation. Water Air Soil Pollut. 105, 95–106.

Likens, G.E., Butler, T.J., Buso, D.C., 2000. Long- and short-term changes in sulfate deposition: effects of the 1990 Clean Air Act Amendments. Biogeochem 52, 1–11.

Llamas, R., 1990. Geomorphology of the aeolian sands of the Doñana National Park (Spain). Catena 18, 145–154 (Suppl.).

López, T., Mazuelos, N., Muñoz-Reinoso, J.C., 1994. Spatial and temporal variation in the ionic composition of shallow water in Doñana National Park (SW Spain). Verh. Int. Ver. Limnol. 25, 1438–1444.

Lozano E. 2004. Las aguas subterráneas en Los Cotos de Doñana y su influencia en las lagunas. PhD thesis. Barcelona: Universidad Politécnica de Barcelona; 2004.

Lucassen, E.C.H.E.T., Smolders, A.J.P., Roelofs, J.G.M., 2002. 2002. Potential sensitivity of mires to drought, acidification and mobilisation of heavy metals: the sediment S/(Ca + Mg) ratio as diagnostic tool. Environ. Pollut. 120, 635–646.

Manzano, M., Custodio, E., Lozano, E., Higueras, H., 2007. Relationships between wetlands and the Doñana coastal aquifer (SW Spain). In: Ribeiro, L., Chambel, A., Condesso de Melo, M.T. (Eds.), XXXV Congress of the International Association of Hydrologists. DTP Solutions, Cape Town, pp. 1–10.

McCullough, C.D., Horwitz, P., 2010. Vulnerability of organic acid tolerant wetland biota to the effects of inorganic acidification. Sci. Total Environ. 408, 1868–1877.

Muñoz-Reinoso, J.C., 2001. Vegetation changes and groundwater abstraction in SW Doñana, Spain. J. Hydrol. 242, 197–209.

Neumann, C., Beer, J., Blodau, C., Peiffer, S., Fleckenstein, J.H., 2013. Spatial patterns of groundwater-lake exchange -implications for acid neutralization processes in an acid mine lake. Hydrol. Process. 27, 3240–3253.

Pierce, B.A., 1985. Acid tolerance in amphibians. Bioscience 35, 239–243.

Räsänen, K., Green, D.M., 2009. Acidification and its effects on amphibian populations. In: Heatwole, H. (Ed.), Decline: diseases, parasites, maladies and pollutionAmphibian biology vol. 8. Surrey Beatty, Chipping Norton, pp. 3244–3267.

Reina, M., Portillo, M.C., Serrano, L., Lucassen, E.C.H.E.T., Roelofs, J.G.M., Romero, A., González, J.M., 2015. The interplay of hydrological, chemical and microbial processes in the formation of iron-rich floating films in aquatic environments at a circumneutral pH. Limnetica 34, 365–380.

Sacks, L.A., Herman, J.S., Konikow, L.F., Vela, A.L., 1992. Seasonal dynamics of groundwater-lake interactions at Doñana National Park, Spain. J. Hydrol. 136, 123–154.

Schorr, M.S., Dyson, M.C., Nelson, C.H., Van Horn, G.S., Collins, D.E., Richards, S.M., 2013. Effects of stream acidification on lotic salamander assemblages in a coal-mined watershed in the Cumberland Plateau. J. Freshw. Ecol. 28, 339–353.

Serrano, L., Serrano, L., 1996. Influence of groundwater exploitation for urban water supply on temporary ponds from the Doñana National Park (SW Spain). J. Environ. Manag. 46, 229–238.

Serrano, L., Toja, J., 1995. Limnological description of four temporary ponds in the Doñana National Park (SW Spain). Arch. Hydrobiol. 133, 497–516.

Serrano, L., Zunzunegui, M., 2008. The relevance of preserving temporary ponds during drought: hydrological and vegetation changes over a 16-year period in the Doñana National Park (south-west Spain). Aquat. Conserv. 18, 261–279.
Serrano, L., Reina, M., Martín, G., Reyes, I., Arechederra, A., León, D., Toja, J., 2006. The aquatic systems of Doñana: watersheds and frontiers. Limnetica 25, 11–32.
Siljeström, P.A., Moreno, A., García, L.V., Clemente, L.E., 1994. Doñana National Park (south-west Spain): geomorphological characterization through a soil vegetation study. J. Arid Environ. 26, 315–323.
Suso, J., Llamas, R., 1993. Influence of groundwater development on the Doñana National Park ecosystems (Spain). J. Hydrol. 141, 239–269.
Takem, G.E., Kuitcha, D., Ako, A.A., Mafany, G.T., Takounjou-Fouepe, A., Ndjama, J., Ntchancho, R., Ateba, B.H., Chandrasekharam, D., Ayonghe, S.N., 2015. Acidification of shallow groundwater in the unconfined sandy aquifer of the city of Douala, Cameroon, Western Africa: implications for groundwater quality and use. Environ. Earth Sci. 74, 6831–6846.
Trick, T., Custodio, E., 2004. Hydrodynamic characteristics of the western Doñana Region (area of El Abalario), Huelva, Spain. Hydrogeol. J. 12, 321–335.
Turner, J.N., Brewen, P.A., Macklin, M.G., 2008. Fluvial-controlled metal and As mobilisation, dispersal and storage in the Río Guadiamar, SW Spain and its implications for long-term contaminant fluxes to the Doñana wetlands. Sci. Total Environ. 394, 144–161.
Valverde, J.A., 1967. Estructura de una comunidad de vertebrados. Monogr. Est. Biol. Doñana 1, 1–218.
Van Geen, A., Adkins, J.F., Boyle, E.A., Nelson, C.H., Palanques, A., 1997. A 120 yr record of widespread contamination of the Iberian Pyrite Belt. Geology 25, 291–294.
Wells, K.D., 2007. The ecology and behavior of amphibians. Chicago University Press, Chicago.
Wiegand, B.A., 2009. Tracing effects of decalcification on solute sources in a shallow groundwater aquifer, NW Germany. J. Hydrol. 378, 62–71.
Woodhams, D.C., Alford, R.A., Briggs, C.J., Johnson, M., Rollins-Smith, L.A., 2008. Lifehistory trade-offs influence disease in changing climates: Strategies of an amphibian pathogen. Ecology 89, 1627–1639.
Zunzunegui, M., Díaz-Barradas, M.C., García-Novo, F., 1998. Vegetation fluctuation in Mediterranean dune ponds in relation to rainfall variation and water extraction. Appl. Veg. Sci. 1, 151–160.