Sea water has a salt concentration of 32 to 37.5‰ (salinity of 35‰ = 10.77 g Na⁺, 0.399 g K⁺, 19.354 g Cl⁻, dissolved in one kilogram of solution; Viertel, 1999); brackish water 0.5 to 32‰; and freshwater 0 to 0.5‰. Amphibians are poorly adapted to survive in saline environments; only a few species are recognized as euryhaline, and there is no marine species (Licht et al., 1975). However, only specific populations of these euryhaline species are adapted to survive in saline environments (Gomez-Mestre and Tejedo, 2003; Ortiz-Santaliestra et al., 2010). Three anurans, the crab-eating frog, *Rana cancrivora*, the European green toad, *Bufo viridis*, and the African clawed frog, *Xenopus laevis*, are among the most tolerant known species to salinity (Balinsky, 1981). In fact, some populations of *R. cancrivora* from Thailand have the highest salt tolerance described in scientific literature, tolerating undiluted sea water (33‰; Ang Sila near Bangkok; Uchiyama and Yoshizawa, 1992). In the wild, this frog can live in waters with salt concentrations between 14 and 28‰, but from fresh to sea water in laboratory conditions. Tadpoles with external gills tolerated up to 15‰ salinity concentration, but died in water of higher salinity; tadpoles with internal gills were able to tolerate 19‰ salt concentration (Uchiyama and Yoshizawa, 1992). *Bufo viridis* adults from northern Europe (Gislén and Kauri, 1959) and Yugoslavia (Gordon, 1962) can resist up to 19‰ salt concentration. Juveniles of *Xenopus laevis* from a population introduced in California survived up to 8‰ salt concentration (Munsey, 1972).

Other example of high salinity tolerance species is the Natterjack toad *Bufo calamita* (Viertel, 1999; Gómez-Mestre and Tejedo, 2003; Gómez-Mestre and Tejedo, 2005). Its upper lethal limit for adults from England in saline water is four days with a salt concentration of 16 to 17‰. However, breeding in Sweden occurred at a salinity of 4‰, while the larvae died up to 7‰ (Viertel, 1999 and references therein). Populations of the small plethodontid salamanders *Batrachoseps relictus* and *B. attenuatus* living in close proximity to sea water on ocean beaches in central California can survive perfectly with salt concentrations of 11‰, while interior populations died in a time period of five days (Licht et al., 1975).

Similarly, individuals of the Southern Leopard frog *Rana sphenocephala* from hypersaline environments in Florida are more tolerant to sea water than other individuals from hyposaline environments (Christman, 1974). The abundance of the Cane toad *Bufo marinus* increases with salinity concentration in wetlands of Puerto Rico, while the abundance of another syntopic species (*Leptodactylus albilatris*) decreases (Rios-Lópex, 2008). At concentrations of 8-9‰ of sea water, no tadpoles of the latter are able to perform metamorphosis. In a similar way, species richness of wetland communities decreases with salinity (Smith et al., 2007).

Less tolerant amphibians are the adults of the Common frog *Rana temporaria* from Germany, which select places with low salinity for spawning (Viertel, 1999). Moderate levels of salinity (6‰) reduce survival and retard development of tadpoles of the Brown Tree frog *Litoria ewingii* from South Australia (Chinatombmy et al., 2006). Tadpoles of the Green and Golden Bell frog *Litoria aurea* from south-western Australia resist up to 1.5‰ of salinity, suffer growth reduction at 2.1‰...
and died almost completely with 3.7‰ (Christy and Dickman, 2002). Hangström (1981) found tadpoles of *Bufo bufo* in brackish waters (3.5‰ of salt concentration) of Baltic sea.

Amphibian species use several physiological mechanisms to avoid osmotic shocks: 1) increasing osmotic pressure inside the organism (through urea synthesis, circulating sodium and chloride increase); 2) reducing surface/volume ratio (i.e. increasing body size) to minimize the relative loss of water per unit mass; and 3) thickening the skin to make the cutaneous water diffusion more difficult (Balinsky, 1981). Adults living in saline environments synthesize more urea in plasma and tissues in order to increase the osmotic pressure of body fluids (Wright et al., 2004). They decreased skin sodium transport and urine flow, and release pituitary hormones posteriorly, causing an increment of urea retention (Balinsky, 1981; Uchiyama and Yoshizawa, 1992). For example, *R. sphenoecephala* reduces water loss in hypersaline environments through concentrating plasma and incrementing body size as well as skin thickness; thus, the frog decrease the osmotic gradient between the frog and the environment, the surface area relative to the internal volume of water, and permeability (Christman, 1974).

We found in autumn 2009 some individuals of *Pelophylax perezi* emitting calls in several small ponds on a rocky coast of Porto (Portugal; Fig 1). After that, we made several systematic surveys with several objectives: first, to confirm the records; second, to count the number of ponds occupied by frogs; and third, to measure the salinity concentration of pond water. We performed five surveys during 2010 (March 3rd, May 9th, May 15th, June 6th and August 19th), finding frogs in the last four surveys (ponds are mapped and numbered in Fig. 2). In the second survey (5/9/2010), we found one adult and one sub-adult in a small pond very close to the seashore (not represented in Fig. 2). The salinity and conductivity of water pond were, respectively, 28‰ and 5.33 mS/cm, i.e. brackish water. The pond was located in an elevated position above the coast, thus the main water source should be the rain; influx of sea water should be small, occurring only in very windy days. There was no vegetation on the pond, which contained a large amount of garbage. In the third survey (5/15/2010), we found again the same number of individuals from the second survey plus eight frogs singing in several ponds (ponds 1 to 4 in Fig 2A). Furthermore, in one pond we found also many small larvae (pond 1 in Fig. 2A and 2C). Almost all these ponds were composed by clean-transparent waters with vegetation (the green alga *Enteromorpha intestinalis*) and marine fishes (*Liza ramada*), and located at different altitudes above the rocky coast. One of the ponds had a direct connection to the sea during high tide (pond 2 in Fig. 2A and 2B). In the fourth survey (6/6/2010), we found nine frogs in four ponds (ponds 1 to 4 in Fig. 2A and 2B). In the last survey (8/19/2010), ponds had very low water levels. We found 20 frogs in four ponds (ponds 1 to 4 in Fig. 2A) and tadpoles in two of these ponds (ponds 1 and 4 in Fig. 2A). Pond 4 had tadpoles in a very

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**Figure 1.** Location maps of the water sea ponds with adults and larvae of *Pelophylax perezi*. Map A: location of Portugal (in bluish colour) and Porto city (red point) in the Iberian Peninsula; map B: city of Porto with the location of water sea ponds (black arrow); and map C: location of the six sea water ponds.
Reproduction of *Pelophylax perezi* in brackish water

advanced state. There was calling activity in the fourth and fifth surveys. We recorded more individuals in the last survey probably due to the lower volume of water, and thus a higher concentration of frogs.

We took two samples of water from two ponds: from pond 1 (Fig. 2A and 2C), the first pond with tadpoles (3‰ of salinity, 1.08 mS/cm of conductivity); and from pond 2 (Fig. 2A), very close to the sea (11‰ of salinity, 2.45 mS/cm of conductivity; Fig. 2B). Therefore, pond water could be considered as brackish. The first pond (second survey) analysed had a higher salinity probably due to a higher evaporation rate, as the pond receives water mainly from rain. The other two ponds analysed received fresh water from very small temporary streams, thus reducing salinity proportion. There were other ponds without frogs, very close to the sea (less than two metres during high tide), and probably with a higher salinity concentration, as the presence of mollusc and crustaceans suggested.

This is the first time that the presence and reproduction of anurans (*P. perezi* in particular) is reported in sea water in the Iberian Peninsula. The most tolerant populations to salinity known so far were *Bufo calamita* (reported in some zones of Madrid; Garcia-Paris, 2004) and *P. perezi* (reported in waters of low salinity in peninsular Spain, Llorente et al., 2002; and in brackish waters with 0.75 g/l of NaCl in the Balearic Islands, Garcia-Paris, 2004).

However, Gomez-Mestre and Tejedo (2003) reported reproduction of *B. calamita* in southern Spain (brackish waters with 0.2 to 22‰ of salinity), and Ortiz-Santíliestra et al. (2010) reported reproduction on brackish lakes (2.04‰ of salinity) in the interior of peninsular Spain. Other Iberian species, like *Pleurodels waltl*, *Triturus marmoratus*, *Pelobates cultripes*, *Pelodytes ibericus*, *P. punctatus*, and *Hyla meridionalis*, are tolerant to low levels of salinity being frequently found in coastal dune systems (García-Paris, 2004). Therefore, this is the first time to our knowledge that reproduction of *P. perezi* is reported on waters with increased salinity due to sea water influence. Further research should be performed in order to estimate the population size and annual use of brackish ponds. As pond salinity can change over time, especially between rain and dry periods, it would be relevant to understand which is the range of the frog’s salinity adaptation, and whether frogs change the use of ponds depending on salinity proportion.

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