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Salinity of the reproduction habitats of the Western Spadefoot Toad *Pelobates cultripes* (CUVIER, 1829), along the Atlantic coast of France
(Anura: Pelobatidae)

Salinität der Laichgewässer des Messerfußes, *Pelobates cultripes* (CUVIER, 1829) entlang der Atlantikküste von Frankreich
(Anura: Pelobatidae)

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KURZFASSUNG

Von 138 entlang der französischen Atlantikküste besammelten Wasserstellen enthielten 50 Kaulquappen von *Pelobates cultripes* (CUVIER, 1829). Die Fortpflanzungsstätten des Messerfußes lagen überwiegend in dünennahen Feuchtgebieten mit Salinitätswerten von unterhalb der Nachweisgrenze (< 1 ‰) bis 10 ‰. Im Zuge der Untersuchungen wurden neben den beobachteten Amphibien und ihren Entwicklungsstadien die maximalen Salinitätswerte und die Makrophytenflora der Gewässer registriert. Physiologische Anpassungen ermöglichten es zahlreichen Amphibienarten, die Salzmarschen zu bewohnen, wie sie früher entlang der französischen Atlantikküste zu finden waren. Vermehrte bauliche Eingriffe im Rahmen von Austern- und Fischzucht bewirkten Veränderungen in der Wassertiefe der Salzmarschen und äußerten sich in einem Trend hin zu einer Vereinheitlichung der Salinität der Wasserkörper mit Angleichung an jene des Seewassers (35 ‰). Für Feuchtgebiete, die nach der 'Habitats Direktive' der Europäischen Union Räume von öffentlichem Interesse darstellen, wird im Hinblick auf den Erhalt eines Salinitätsgradienten ein hydrologisches Management gefordert.

ABSTRACT

Out of 138 aquatic habitats sampled along the French Atlantic coast, 50 contained tadpoles of *Pelobates cultripes* (CUVIER, 1829). These reproduction habitats of the Western Spadefoot, mainly located in wetland areas near dunes, were characterized by salinity values varying from below detection limit (< 1 ‰) to 10 ‰. During the survey, additional amphibian species observed in the aquatic sites were registered along with the water bodies' maximum salinity values and macrophyte flora. Physiological adaptations to salinity allowed numerous species of amphibians to live in the salt marshes once present along the Atlantic coast. Recent increase of anthropogenic activities such as fish and oyster farming produced changes in the water depth of these marshland systems, with a tendency towards homogenized salinity levels, more and more resembling that of seawater (35 ‰). For those wet zones which, according to the implementation of the 'Habitats Directive' of the European Union, represent sites of public concern, hydrological management is to be demanded focusing on the maintenance of a salinity gradient.

KEY WORDS

Amphibia: Anura: Pelobatidae; *Pelobates cultripes*, Western Spadefoot Toad, reproduction habitats, salinity, ecology, physiology, Atlantic coast, anthropogenic threats, France

INTRODUCTION

Most amphibians are intolerant to salinity levels exceeding usual freshwater values (DUELLMAN & TRUEB 1994; WELLS 2007). However, bibliography mentions the reproduction of European species in brackish habitats for *Pleurodeles waltl* MICHAHELLES, 1830, *Triturus cristatus* (LAURENTI, 1768), *Triturus marmoratus* (LATREILLE, 1800), *Lissotriton helveticus* (RAZOUMOVSKY, 1789), *Lissotriton vulgaris* (LINNAEUS, 1758), *Discoglossus galganoi* CAPULA, NASCETTI, LANZA, BULLINI & CRESPO, 1985, *Discoglossus pictus* OTTH, 1837, *Discoglossus sardus* TSCHUDI, 1837, *Pelobates cultripes* (CUVIER, 1829), *Pelobates fuscus* (LAURENTI, 1768), *Pelodytes ibericus* SÁNCHEZ-HERRAÍZ, BARBADILLO-ESCRIVÁ, MACHORDOM & SANCHÍZ, 2000, *Pelodytes punctatus* (DAUDIN, 1802), *Bufo bufo* (LINNAEUS, 1758), *Bufo calamita* (LAURENTI, 1768), *Bufo viri-*

Table 1: Literature review about maximum salinity levels tolerated by European amphibians in their aquatic habitat. A – adult, E – eggs, L – larva, S – spawn. [1)] – 23 rarely.

Tab. 1: Liteaturzusammenstellung über den von Europäischen Amphibien im Gewässer maximal tolerierten Salzgehalt (Salinität). A – Adultus, E – Eier, L – Larve, S – Laich. [1)] – 23 selten.

Taxon	Developmental stage / Entwicklungsstadium	Salinity [‰] / Salinität [‰]	Type of study / Untersuchungsart	Source / Quelle
Bufo bufo	L	2.9	Experimemt	MATHIAS (1971)
Bufo bufo	A	13	Experiment	MATHIAS (1971)
Bufo bufo	L	3.5	Observation	HANGSTRÖM (1981)
Bufo calamita	L	2.9	Experiment	MATHIAS (1971)
Bufo calamita	A	17	Experiment	MATHIAS (1971)
Bufo calamita	S, L	3.5	Observation	CURRY-LINDAHL (1975)
Bufo calamita	S, L	4	Observation	ANDRÉN & NILSON (1985)
Bufo calamita	S	4.2	Observation	BEEBEE (1985)
Bufo calamita	L	7	Observation	BEEBEE (1985)
Bufo calamita	A	13.5	Observation	SINSCH et al. (1992)
Bufo calamita	S	10	Observation	GÓMEZ-MESTRE & TEJEDO (2003)
Bufo calamita	S	10	Experiment	GÓMEZ-MESTRE & TEJEDO (2003)
Bufo calamita	L	< 10	Experiment	GÓMEZ-MESTRE & TEJEDO (2003)
Bufo calamita	L	18	Observation	GOMEZ-MESTRE et al. (2004)
Bufo viridis	A	20	Observation	GISLÉN & KAURI (1959)
Bufo viridis	A	19-23[1)]	Experiment	GORDON (1962)
Discoglossus pictus	S	6.8	Observation	KNOEPFFLER (1962)
Discoglossus pictus	L	8	Observation	ACEMAV et al. (2003)
Pelobates fuscus	L	4	Experiment	STĂNESCU et al. (2013)
Pelophylax perezi	S	0.75	Observation	MARGALEF (1951)
Pelophylax perezi	L	3	Observation	SILLERO & RIBEIRO (2010)
Pelophylax perezi	A	28	Observation	SILLERO & RIBEIRO (2010)
Triturus cristatus	E	4	Experiment	WALLACE (1991)

dis (LAURENTI, 1768), *Hyla sarda* (DE BETTA, 1853), *Hyla meridionalis* (BOETTGER, 1874), *Rana temporaria* (LINNAEUS, 1758), *Pelophylax perezi* (LÓPEZ-SEOANE, 1885) and *Pelophylax ridibundus* (PALLAS, 1771) (VALVERDE 1960; HAGSTRÖM 1981; LAHLOU 1995; LLORENTE et al. 1995; BARBADILLO et al. 1999; VIERTEL 1999; ARNOLD & OVENDEN 2002; SPELLERBERG 2002; ACEMAV et al. 2003; GALAN-REGALADO 2003; NÖLLERT & NÖLLERT 2003; REQUES 2003; GARCIA-PARIS et al. 2004; WELLS 2007; SILLERO & RIBEIRO 2010). Precise information on the salinity levels recorded was however, not provided therein. Thus, according to NÖLLERT & NÖLLERT (2003), *P. waltl* tolerates light salinity. Similarly *H. meridionalis* can bear slightly brackish water (ACEMAV et al. 2003) like *T. cristatus* (NÖLLERT & NÖLLERT 2003) or *T. marmoratus* (ACEMAV et al. 2003). As for *P. fuscus*, NÖLLERT & NÖLLERT (2003) point out that the species bears variations in salinity pretty well. Studies mentioning salinity rates tolerated by European amphibians species are rare and only few observations were made in the natural environnement. (Table 1). The study by DIAZ-PANIAGUA (1982) on the factors determining the reproduction habitat of the Western Spadefoot in the Doñana National Park (Andalusia, Spain) is the only publication which reports the rates of salinity which the species can tolerate.

In western France, habitats of *P. cultripes* are mainly located at the coast. However, the high salinity of potential reproduction habitats obviously prevents *Pelobates* from colonizing coastal natural sites. To understand this phenomenon, the author planned to systematically analyze the salinity of reproduction habitats of the Western Spadefoot along the French Atlantic coast and determine the threshold value beyond which tadpoles are no longer present. In addition, co-occurring species of amphibians present in the aquatic habitats used by the Western Spadefoot were registered.

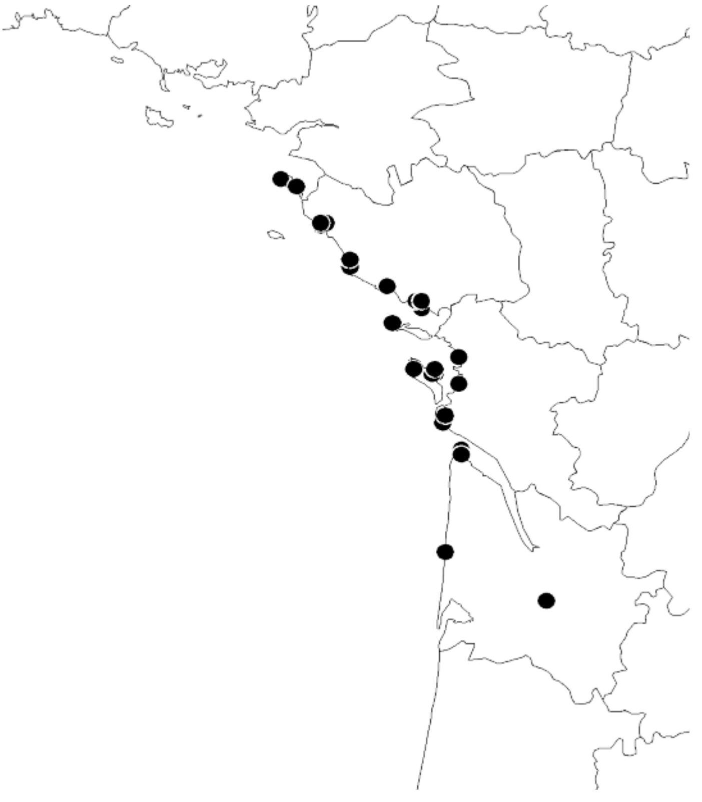

Fig. 1: The record localities of *Pelobates cultripes* (CUVIER, 1829) along the French Atlantic coast, based on data from 1996 to 2001 (THIRION 2006).

Abb. 1: Die Fundorte von *Pelobates cultripes* (CUVIER, 1829) entlang der französischen Atlantikküste auf Grundlage der Erhebungen von 1996 bis 2001 (THIRION 2006).

MATERIALS AND METHODS

Selected characteristics of 138 potential reproduction habitats along the French Atlantic coast and the presence/absence of *P. cultripes* (Fig. 1) were recorded. The reproduction of the Western Spadefoot Toad was evidenced by netting its tadpoles, trying to take samples every 15 minutes and every 10 meters, with one visit per site. For each aquatic habitat visited, the salinity and the aquatic macrophyte vegetation were noted, along with the co-occurring amphibian species. Prospecting was made during the period of presence of the tadpoles, from late May to early June in the years 1998, 1999, 2000 and 2001.

Salinity was taken with an ATAGO automatic temperature compensating refractometer, model ATC-S/Mill-E, with a salinity range of 0.0 to 100.0 ‰ (graduation 1.0 ‰). Between each reading, the refractometer was calibrated. The classes of salinity were determined using the Venice classification of marine waters (VENICE SYSTEM 1959). The type of hydrosystem was defined by the terms of: pond, lagoon, pool, ditch, meadow or dune depression, low marsh and salt marsh depression (“jas“). The “jas“ characterizes a depression corresponding to what used to be the seawater reservoir of a salt marsh.

Table 2: Assessment of the reproduction habitats of *Pelobates cultripes* (CUVIER, 1829) along the French Atlantic coast including aquatic macrophyte species and syntopic amphibians observed. Jas – Depression corresponding to what used to be the seawater reservoir of a salt marsh.

Tab. 2: Die Laichgewässer von *Pelobates cultripes* (CUVIER, 1829) entlang der französischen Atlantikküste, samt den in ihnen festgestellten aquatischen Makrophyten- und Amphibienarten. Jas – Salzwassersenken in ehemaligen Salzmarschen.

H - *Hyla meridionalis* (larvae), Ppu - *Pelodytes punctatus* (larvae), Pes-*Pelophylax* kl. *esculentus* (adults), Ppe - *Pelophylax perezi* (larvae), B - *Bufo calamita* (larvae), T - *Triturus marmoratus* (adults), L – *Lissotriton helveticus* (adults). *Af - Azolla filiculides*, *Cd - Ceratophyllum demersum*, *Ch - Chara* sp., *Gd - Groenlendia densa*, *Gf - Glyceria fluitans*, *Ja - Juncus acutus*, *Ma - Mentha aquatica*, *Ms - Myriophyllum spicatum*, *Of - Oenanthe fistulosa*, *Pa - Phragmites australis, Pp - Potamogeton pectinatus*, *Rb - Ranunculus baudotii*, *Ro - Ranunculus ophioglossifolius*, *Sm - Scirpus maritimus, Tl - Typha latifolia*, *Zp - Zannichellia palustris*.

Department - District	Salinity [‰] / Salinität [‰]	Aquatic macrophytes / Aquatische Makrophyten	Sympatric amphibians / Sympatrische Amphibien	Aquatic system / Gewässertyp
17 - Saint Pierre d'Oléron	9	*Sm*, *Rb*	Ppu, H	Jas
17 - Saint Pierre d'Oléron	2	*Sm*, *Rb*	Ppu, H	Jas
17 - Saint Pierre d'Oléron	4	*Sm*, *Rb*	Ppu, H	Jas
17 - Saint Pierre d'Oléron	2	*Sm*, *Rb*	Ppu, H	Jas
17 - Saint Pierre d'Oléron	4	*Sm*, *Rb, Pp*	Ppu, H	Jas
17 - Saint Clément des Baleines	2	*Sm*, *Rb*	B, Ppu, H, Ppe	Jas
17 - Saint Clément des Baleines	3	*Sm*, *Rb*	Ppu, H	Jas
17 - Saint Clément des Baleines	2	*Pa*, *Cd*	H, Ppe	Meadow pond
17 - Saint Clément des Baleines	1	*Pa*, *Rb*	B, Ppu, H, Ppe	Jas
17 - Saint Denis d'Oléron	<< 1	*Ja*, *Ma*	L, Ppu, Ppe	Dune hollow
17 - La Tremblade	<< 1	*Gf*	L, H	Meadow pond
17 - La Tremblade	<< 1	*Gd*	L, T, H, Ppe,	Meadow pond
17 - La Tremblade	<< 1	*Ch*	L, T, H, Ppe,	Forest pond in dune
17 - Moëze	1	*Ch*	H, Ppe	Temporary dune pond
17 - Moëze	2	*Pp*	Ppu, H, Ppe	Leisure pond
17 - Yves	2	*Sm*	Ppu, H	Temporary meadow pond
17 - Yves	5	*Sm*, *Rb*	Ppu, H, Ppe	Temporary lagoon
17 - Yves	6	*Sm*, *Rb*	Ppu, H, Ppe	Temporary pond
17 - Yves	5	*Sm*, *Rb*	Ppu, H, Ppe	Temporary pond
17 - Yves	4	*Sm*, *Rb*	Ppu, H, Ppe	Temporary pond
17 - Yves	4	*Sm*, *Rb*	Ppu, H, Ppe	Temporary pond
17 - Yves	10	*Sm*	-	Temporary lagoon
17 - Yves	3	*Sm*, *Rb*	Ppu, H, Ppe	Temporary pond
17 - Yves	5	*Sm*, *Rb*	Ppu, H, Ppe	Temporary pond
17 - Yves	5	*Sm*, *Rb*	Ppu, H, Ppe	Temporary pond
17 - Yves	4	*Zp*, *Rb*	Ppu, H, Ppe	Temporary pond
17 - Yves	2	*Pp*	Ppu, H, Ppe	Marsh ditch
17 - Yves	2	*Af*	Ppu, H, Ppe	Marsh ditch
17 - Yves	6	*Sm*, *Rb*, *Pp*	Ppu, H	Temporary lagoon
17 - Yves	1	*Pa*	T, H	Low marsh
85 - Saint-Gilles-Croix-de-Vie	1	*Pp*, *Ms*	Ppu	Marsh ditch
85 - Saint-Gilles-Croix-de-Vie	1	*Of*, *Ro*	Ppu	Meadow hollow
85 - Noirmoutier-en-l'île	1	*Sm*	Ppu	Meadow hollow
85 - Noirmoutier-en-l'île	10	*Sm*, *Rb*	-	Jas
85 - Noirmoutier-en-l'île	1	*Sm*, *Rb*	Ppu	Meadow pond
85 - Noirmoutier-en-l'île	1	*Sm*, *Tl*	L, Pes	Dune pond
85 - La Faute-sur-Mer	5	*Pa*, *Sm*, *Rb*	Ppu	Lagoon
85 - La Faute-sur-Mer	3	*Pa*, *Sm*, *Rb*	Ppu	Lagoon
85 - La Faute-sur-Mer	4	*Sm*	Ppu	Lagoon
85 - La Faute-sur-Mer	5	*Zp*, *Pp*, *Rb*	Ppu	Lagoon
85 - La Faute-sur-Mer	6	*Zp*, *Pp*, *Rb*	Ppu	Lagoon
85 - La Tranche	6	*Sm*, *Pp*, *Rb*	Ppu	Lagoon
85 - La Faute-sur-Mer	7	*Sm*, *Pp*, *Rb*	Ppu	Lagoon
85 - Grues	<< 1	*Lm*, *Gf*	L	Meadow pond
85 - Grues	2	*Sm*, *Pp*, *Cs*	Ppu	Meadow pond
85 - Grues	1	*Sm*, *Lm*	-	Meadow pond
85 - Grues	<< 1	*Gf*	L	Marsh ditch
33 - Verdon-Sur-Mer	3	*Pa*	H, Ppu, Ppe	Jas
33 - Verdon-Sur-Mer	1	*Ch*	H	Artificial pond
33 - Cadaujac	<< 1	*Gf*	L, Pes	Pond

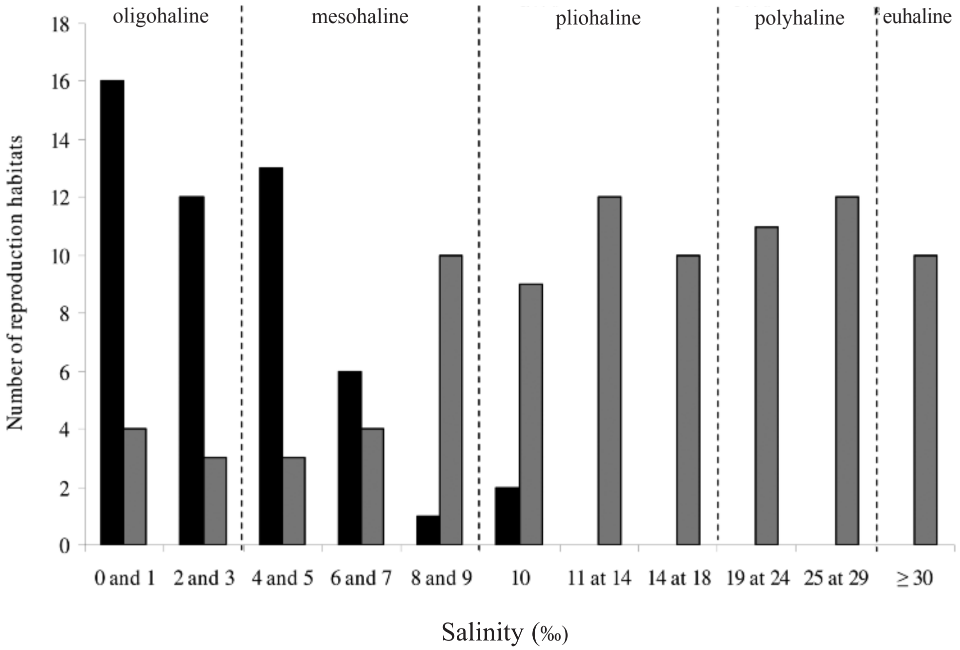

Fig. 2: Number of aquatic habitats in the study area containing (black) or lacking (grey) tadpoles of *Pelobates cultripes* (CUVIER, 1829), grouped according to the salinity (‰) of the water bodies.

Abb 2: Anzahl der Gewässer mit (schwarz) und ohne (grau) Larven von *Pelobates cultripes* (CUVIER, 1829), gruppiert nach der Salinität (‰) der Gewässer.

RESULTS

Salinity: In 50 out of 138 sites sampled, tadpoles of the Western Spadefoot Toad were observed (Table 2). For reproduction, *P. cultripes* used meadow or dune ponds, meadow or dune depressions, coastal lagoons, ponds used for fishing or sailing, ditches in marshes, low alkaline marshes as well as "jas". The reproduction habitats were characterized by salinity values varying from below detection limit (< 1 ‰) to 10 ‰, with a median of 2.5 ‰. Presence/absence of the Western Spadefoot negatively correlated with the salinity of the reproduction habitats (Fishers's exact test; $\chi^2 = 128.59$; $df = 10$; $p < 0.05$). Fourty-eight of 50 reproduction habitats (96 %) were oligohaline and mesohaline, 32 % of which ($N = 16$) contained fresh water from below detection limit (< 1 ‰) to 1 ‰, and 64 % ($N = 32$) brackish water with salinity values of 2 to 9 ‰ (Fig. 2). Two sites (2 %) showed a salinity of 10 ‰. No reproduction site of the Western Spadefoot was observed to hold water of higher salinity values.

Syntopic species: Tadpoles of *Hyla meridionalis* and *Pelodytes punctatus* were observed at a maximum salinity of 9 ‰, whereas those of *Pelophylax perezi* were not found in waters exceeding 6 ‰ (Table 2). On the Isle of Ré (off the coast of the Département Charente-Maritime), the tadpoles of *Bufo calamita* were found in aquatic habitats with a salinity of 2 ‰. Adult *Triturus marmoratus* were seen at 4 ‰ in a former salt marsh near La Rochelle (Table 2).

DISCUSSION

On the French Atlantic coast, the reproduction of the Western Spadefoot Toad was found to take place in partially brackish waters where salinity varied from 0 to 10 ‰. The salinity threshold seemed to be situated at about 10 ‰. Beyond that threshold, the presence of tadpoles was never observed by the author. These results are in agreement with findings in the Doñana National Park (Spain), where the Western Spadefoot Toad did also tolerate an unspecified concentration of salt (DIAZ-PANIAGUA 1982); the species was abundant in brackish watering places where the number of larvae of other species was highly reduced (DIAZ-PANIAGUA 1982).

Overall, aquatic habitats of anurans span a wide range of salinity (MCDIARMID & ALTIG 1999) with the tolerance to salinity varying with species and life history stage (BEEBEE 1996). For example, the best known euryhaline amphibian is *Fejervarya cancrivora* (GRAVENHORST 1829) which lives in the brackish ponds and mangroves of south-east Asia. The tadpoles of this species were observed in aquatic environments where salinity varied from 5 to 24 ‰ and tolerated salinity values of 39 ‰ (BEEBEE 1996) with a survival rate of the larvae more and more reduced with the increase in salinity (DUELLMAN & TRUEB 1994). The present study showed that tadpoles of the Western Spadefoot Toad were observed in water bodies up to a maximum salinity of 10 ‰. An experimental study concerning the development of eggs of this species showed that development stopped at a salinity level of 6 ‰ (THIRION 2006). Cyclone "Martin" of December, 1999 generated a tidal wave which flooded the whole Yves Marsh Natural Reserve where adults of the Western Spadefoot survived salinity levels of 35 ‰ (THIRION 2002). In an experimental study, MATHIAS (1971) showed that the tolerance towards salinity of the tadpoles of *B. bufo* and *B. calamita* was limited to 2.9 ‰ and 4.3 ‰ respectively, whereas adults managed 13 ‰ and 16 ‰, respectively (cf. Table 1). Lowered salinity tolerance of tadpoles is explained by their mode of excretion. Adult amphibians are ureotelic whereas the larvae are ammonotelic, accumulating and eliminating metabolic waste products in the form of inorganic ions (LAHLOU 1995). Thus, increased concentrations of inorganic ions in the ambient water are likely to overstrain or impair the elimination performance of the larval excretory system. Some phenomena remain however, insufficiently understood: The salinity tolerance of tadpoles varied according to their developmental stage, where earlier stages were more tolerant (VIERTEL 1999), and tadpoles living in brackish waters were more tolerant to salinity variation than tadpoles of freshwater habitats possibly indicating some genetic adaptation of these populations relative to this type of environment (GOMEZ-MESTRE & TEJEDO 2003, 2004; WELLS 2007).

As shown in the introduction, many European amphibians can use brackish habitats, while their limits of tolerance to salinity in the aquatic environment are unknown. Additional studies are required to improve our knowledge on amphibian salinity tolerance at different stages of development in nature.

Between the estuary of the Gironde River and the Department of Vendée, dunes lining those former salt marshes are preferred habitats of the Western Spadefoot Toad (THIRION 2006). The general decline in salt production around 1830 in parts of those marshes certainly favored rates of salinity below or equal to 10 ‰ (e.g., CORLAY 1986). Between 1990 and 2000, monitoring of anthropogenic activities in French Atlantic marshes indicated a slight but significant increase of pressure on natural environments (XIMENÈS et al. 2007). The increase of anthropogenic activities such as fish and oyster farming produced changes in the water depth of these marshland systems with a tendency towards homogenized salinity levels, more and more resembling that of seawater (35 ‰), leaving less fresh water or slightly brackish areas (e.g., GUEZEL et al. 2006). These changes may result in fragmentation of the Western Spadefoot's breeding habitats. For those wet zones, which, according to the implementation of the

'Habitats Directive' of the European Union, represent sites of common European interest, hydrological management is to be demanded as to the maintenance of a salinity gradient.

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