



# Can stable isotopes assess habitat use in complex coastal wetlands? A case study in an amphibian species

Léa Lorrain-Soligon<sup>a,\*</sup>, Frédéric Robin<sup>b,c,d</sup>, Vincent Lelong<sup>c</sup>, Marko Jankovic<sup>d</sup>,  
Paco Bustamante<sup>e,f</sup>, Yves Cherel<sup>a</sup>, François Brischoux<sup>a</sup>

<sup>a</sup> Centre D'Etudes Biologiques de Chizé (CEBC), UMR 7372 Du CNRS – La Rochelle Université, 79360, Villiers-en-Bois, France

<sup>b</sup> LPO France, Fonderies Royales, 17300, Rochefort, France

<sup>c</sup> Réserve Naturelle de Moëze-Oléron, LPO, Plaisance, 17780, Saint-Froult, France

<sup>d</sup> Réserve Naturelle Du Marais D'Yves LPO, Ferme de La Belle Espérance, 17340, Yves, France

<sup>e</sup> Littoral Environnement et Sociétés (LIENSs), UMR 7266 CNRS-La Rochelle Université, 2 Rue Olympe de Gouges, 17000, La Rochelle, France

<sup>f</sup> Institut Universitaire de France (IUF), 1 Rue Descartes, 75005, Paris, France

## ARTICLE INFO

### Keywords:

Isotopic niche  
Osmolality  
*Pelobates cultripes*  
 $\delta^{15}\text{N}$   
 $\delta^{13}\text{C}$   
 $\delta^{34}\text{S}$

## ABSTRACT

Coastal ecosystems are among the most diverse and productive systems on earth, but they are threatened by various anthropogenic pressures. To understand the magnitude of these constraints on biodiversity, it is essential to assess habitat use of coastal species, which can be challenging in the complex matrix of habitats in coastal environments. The stable isotope method is a powerful tool to assess foraging habitat, as marine influences on the trophic web should decrease with increasing distance from the ocean. In this study, we evaluated whether isotopic values ( $\delta^{15}\text{N}$ ,  $\delta^{13}\text{C}$  and  $\delta^{34}\text{S}$ ) can be useful to assess habitat use in a coastal amphibian, the western spadefoot toad (*Pelobates cultripes*). In two coastal wetlands of the French Atlantic coast, we captured individuals from 20 to 1300 m from the seashore in order to assess whether isotopic values were related to the distance to the coastline, and we combined this dataset with data on osmolality that correlate to distance to the ocean in this species. We found divergent site-specific responses, presumably linked to the site-specific extent of a recent marine submersion, highlighting the long-lasting effects of such extreme weather event on trophic webs. In both sites however, we found a larger isotopic niche closer to the seashore suggesting wider diet or prey depending on wider resources near the sea. Overall, our results suggest that isotopic values can provide insights on habitat use depending on site-specific characteristics. Combining isotopic values with other metrics (e.g., osmolality) allows to better understand isoscapes in a site-specific context.

## 1. Introduction

Terrestrial coastal ecosystems are located at the boundary between oceanic and terrestrial ecosystems. They are one of the most dynamic interfaces among biomes (Sheaves, 2009) and are subject to complex interactions between marine and terrestrial influences (McLean et al., 2001). As a consequence, coastal ecosystems are among the most diverse and productive systems on earth (Hobohm et al., 2021; Sheaves, 2009). They are recognized as hotspots of biodiversity providing resources for a large number of species (Hobohm et al., 2021). In addition, they contribute to a large panel of ecosystem services (Barbier, 2015; Spalding et al., 2014). Yet, they are threatened by various anthropogenic activities such as urbanization, agriculture, environmental

contamination, tourism and overexploitation (Hobohm et al., 2021). In addition to these spatially restricted threats, coastal areas are also predicted to be impacted by climate change through two main processes: the progressive rise of sea level (Kulp and Strauss, 2019) and the increase in frequency and intensity of storms and associated marine submersions (i.e., causing ocean water to temporary cover coastal areas, Dettinger, 2011; McLean et al., 2001). All of these threats are expected to affect coastal biodiversity, but it remains essential to assess habitat use of coastal species to better understand how increasing environmental changes affect coastal ecosystems and their biodiversity.

Coastal wetlands are composed of a complex matrix of habitat types distributed across very short spatial scales (e.g., few hundred meters, McLean et al., 2001). These habitats cover a continuum between

\* Corresponding author. Centre d'Etudes Biologiques de Chizé (CEBC), 405 route de Prissé la Charrière, 79360, Villiers-en-Bois, France.

E-mail address: [lea.lorrain-soligon@cebc.cnrs.fr](mailto:lea.lorrain-soligon@cebc.cnrs.fr) (L. Lorrain-Soligon).

<https://doi.org/10.1016/j.ecss.2022.107953>

Received 18 February 2022; Received in revised form 9 June 2022; Accepted 14 June 2022

Available online 16 June 2022

0272-7714/© 2022 Elsevier Ltd. All rights reserved.

intertidal sandy area, mudflats and seagrass beds; through salt and freshwater marshes or swamps; up to sand dunes, grasslands and forests; along with increasing distance from the seashore (Maynard and Wilcox, 1997; McLean et al., 2001). In most cases, a given species will use each of these habitat types differentially according to its life-history stage (Arthur et al., 2008; Rathbun et al., 2002), its physiological state (Brischoux et al., 2013) and its energetic requirements (Schwemmer and Garthe, 2008). Foraging habitat is thus a central feature of overall habitat use as it will determine the quality and the quantity of resources available to an organism, which will be selected according to life stage of an individual (growth, reproduction, migration); or individual strategies and dietary specialization (Robinson and Wilson, 1998). In addition, foraging is often associated with risk-taking behaviours (Verdolin, 2006). These elements suggest that precise selection of a suitable foraging habitat will ultimately influence fitness through increased energetic incomes and reduced predation risks. Importantly, in coastal ecosystems, the use of habitats situated close to the seashore is also expected to reflect an individual's vulnerability to extreme weather events (e.g. marine submersions, Lorrain-Soligon et al., 2021) and thus the persistence of coastal populations. Yet, assessing the habitat use of individuals within the complex matrix of habitats highlighted above is a challenging task that may reveal logistically prohibitive (e.g., fine scale tracking of individual movements, Ponchon et al., 2013). The use of stable isotopes analyses can offer a powerful alternative to identify hard-to-measure variables, such as foraging habitats (Bearhop et al., 2003; Choy et al., 2011; Monti et al., 2021).

The concept of the isotopic niche is based on the fact that an individual's chemical composition is influenced by what it consumes and assimilates (Brüssow and Parkinson, 2014; Kohn, 1999), which can be characterized by analyzing stable isotopes (Kudman, 2021; Layman et al., 2012; McCue et al., 2020). Stable nitrogen isotope values ( $\delta^{15}\text{N}$ ) are mostly used as a surrogate of trophic position, but can be also a relevant proxy of consumers' foraging habitat (Minagawa and Wada, 1984; Peterson and Fry, 1987; Vander Zanden and Rasmussen, 1999). In our context,  $\delta^{15}\text{N}$  is thought to increase with increasing salinity (Fry, 2002; Hussain and Al-Dakheel, 2018), and may thus increase in marine compartments compared to terrestrial ones. Complementarily, stable carbon values ( $\delta^{13}\text{C}$ ) vary among specific primary producers and this parameter can be used to examine differences in trophic support and thus presumably habitats (Minagawa and Wada, 1984; Peterson and Fry, 1987; Vander Zanden and Rasmussen, 1999). Similarly to  $\delta^{15}\text{N}$ ,  $\delta^{13}\text{C}$  is thought to increase with salinity (Fry, 2002), and thus values for oceanic carbon should be higher than for terrestrial one. Finally, stable sulfur values ( $\delta^{34}\text{S}$ ) allow discriminating between marine or terrestrial food webs since values for marine sulfate are generally higher (Leyden et al., 2021; Lott et al., 2003; Michener, 1994). As such, like for  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values,  $\delta^{34}\text{S}$  is thought to decrease when salinity decreases and distance from the coastline increases (Fry and Chumchal, 2011).

Isotopic values of all three elements are expected to vary across coastal habitats, especially according to increasing distance from the ocean. Indeed, coastal winds cause landward transport of matter such as sediment (Ridderinkhof, 1998; Zhang et al., 2020), organic matter (Pelegrí et al., 2005) and salts (Demoisson et al., 2013); and their deposition is expected to progressively decrease with increasing distance from the shore (Meira et al., 2008; Mustafa and Yusof, 1994). Accordingly, the influence of such deposition of matter of marine origin on the trophic web – and thus on stable isotope values – should progressively decrease with increasing distance from seawater. It is therefore expected that isotopic values will be enriched close to the seashore, and that they will decrease with increasing distance from the ocean. Indeed, an individual foraging closer to the seashore should display  $\delta^{15}\text{N}$ ,  $\delta^{13}\text{C}$  and  $\delta^{34}\text{S}$  values close to those from marine systems, while individuals foraging further inland should display  $\delta^{15}\text{N}$ ,  $\delta^{13}\text{C}$  and  $\delta^{34}\text{S}$  values indicative of terrestrial ecosystems.

In this study, we tested this hypothesis and evaluated whether  $\delta^{15}\text{N}$ ,  $\delta^{13}\text{C}$  and  $\delta^{34}\text{S}$  values can be useful to assess habitat use (proximity to the

seashore) in the terrestrial coastal amphibian *Pelabates cultripes* captured along a continuum of distance from the seashore (from 20 to 1300 m). Because landward transport of sediments and organic matters occurs primarily at a very short spatial scale (Bainbridge et al., 2018; Ridderinkhof, 1998), we also investigated these variations at a smaller extent, within 200 m from the seashore. Such decrease in marine influence on isotopic values has already been suggested with a similar variation of osmolality of toad blood according to distance to seawater (Lorrain-Soligon et al., 2022), because osmolality is related to salt exposure through sea-spray (Benassai et al., 2005; McLean et al., 2001). As a consequence, we complemented our isotopic analyses with osmolality measurements (Lorrain-Soligon et al., 2022) as an independent marker of marine influence and we predicted that individual's plasma osmolality should correlate with  $\delta^{15}\text{N}$ ,  $\delta^{13}\text{C}$  and  $\delta^{34}\text{S}$  values.

## 2. Materials and methods

### 2.1. Study species

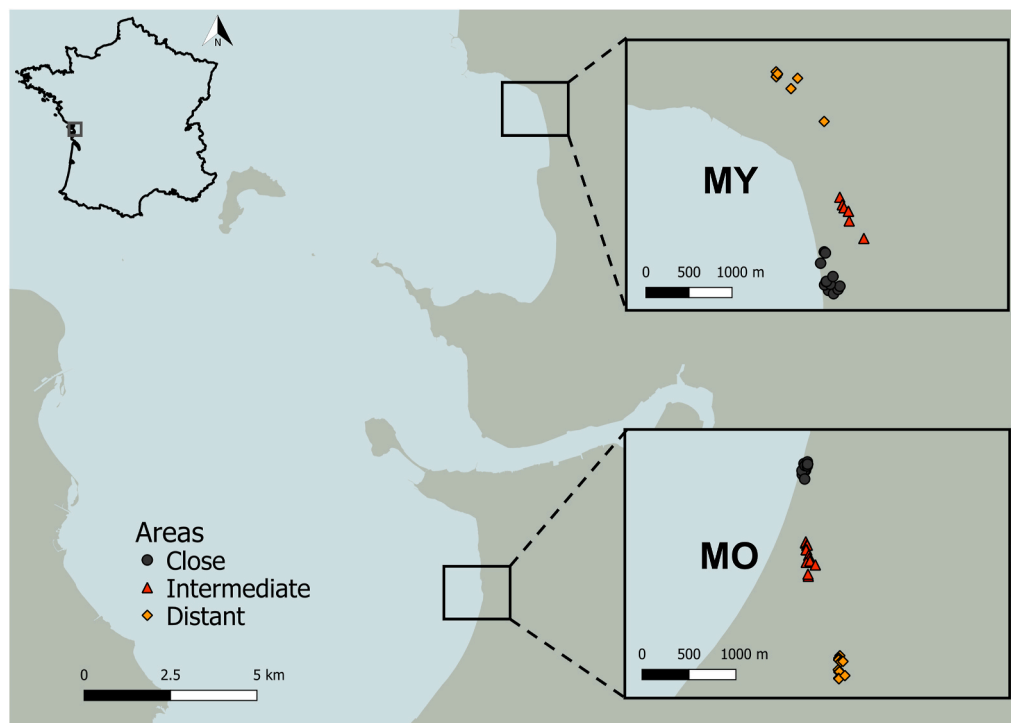
The western spadefoot toad, *Pelabates cultripes*, is an amphibian relatively tolerant to salinity (Stănescu et al., 2013; Thirion, 2014). This species is mostly distributed in the region of the Iberian Peninsula and along the French Mediterranean and Atlantic coasts (Lizana et al., 1994; Leclair et al., 2005; Thirion, 2014). As most amphibians, western spadefoot toads have a biphasic lifestyle. Eggs and larvae develop in fresh and brackish water bodies (Stănescu et al., 2013), while terrestrial juveniles and adults are distributed from very close to the seashore up to 5 km inland, where they shelter in sandy soils during the day and forage for invertebrates at night (Speybroeck et al., 2018).

### 2.2. Study sites

The study was carried out from October 7, 2020 to October 23, 2020 on two natural reserves located on the Atlantic coast of France (Département de la Charente-Maritime), and separated by ~15 km straight line: the « Réserve Naturelle Nationale de Moëze-Oléron » (45°54'20.4"N, 1°04'19.9"W, hereafter MO), and the « Réserve Naturelle Nationale du marais d'Yves » (46°2'40.735"N, 1°3'16.906"W, hereafter MY) (see Fig. 1). The two sites have been thoroughly described in Lorrain-Soligon et al. (2021), including their topographic profiles. Both sites are low coastal wetlands (mean elevation: MY = 2.64 m  $\pm$  0.02 SE; MO = 2.90 m  $\pm$  0.01 SE), with sand dunes near the seashore (~100 m from the seashore; 3.30 m high for both sites). Additionally, MO displays an additional dune situated ~500 m inland. These slightly different topographies have led to different consequences of marine submersions. Indeed, both sites were hit by storm Xynthia in 2010, during which MY was completely submerged, while the extent of the submersion was minor in MO (see Lorrain-Soligon et al., 2021). These two sites are composed of a matrix of habitats typical of coastal wetlands: salt marsh, meadows, and freshwater ponds. In MO, pond salinity decreases with increasing distance from the shore, while in MY, pond salinity remains constant (Lorrain-Soligon et al., 2022). Both sites are exposed to an oceanic climate (Cfb according to Köppen classification), and to the same regime of winds.

### 2.3. Field procedures

On each site, three areas were prospected according to their distance to the seashore: close (<200 m from the ocean), intermediate (between 200 and 600 m to the ocean), and distant (>600 m from the ocean) (see Fig. 1), all characterized by the presence of sandy soil. All field surveys were carried out at night, between 9 p.m. and 4 a.m. Sixty-seven adults were captured (35 in MO, 28 females and 7 males, and 32 in MY, 24 females and 8 males). Each individual toad was measured (Snout-to-Vent Length [SVL]) using a caliper [ $\pm 1$  mm]), weighed (pesola spring scale [ $\pm 0.5$  g]), and sexed using secondary sexual characters



**Fig. 1.** Map of Western France including the two study sites and the position of the captured *Pelobates cultripes* in the close, intermediate and distant areas from the shore at each site. MO: Réserve Naturelle Nationale de Moëze-Oléron, and MY: Réserve Naturelle Nationale du marais d'Yves.

(Speybroeck et al., 2018). Characteristics of body mass and size of the individuals are given in Appendix A. Precise locations of capture were recorded with a GPS (Garmin GLO) to assess the exact position of individuals, and thus its distance to the ocean at high tide, using the NNJoin extension on Qgis 3.10.

All blood sampled individuals weighted  $>15$  g. Blood was collected by cardiocentesis, and blood volume was  $\leq 150$   $\mu$ l, thus representing  $<1\%$  of toad body mass (Diehl et al., 2001; Soulsbury et al., 2020). Blood was centrifuged for 7 min at 2000 G to separate plasma from blood cells. Both fractions were stored frozen at  $-20$  °C until analyses. Plasma osmolality ( $\text{mOsmol.kg}^{-1}$ ) were re-analyzed from Lorrain-Soligon et al. (2022) and red blood cells (hereafter blood) were used for isotopic analyses (see below).

#### 2.4. Isotopic analyses

Isotopic analyses were carried out on freeze-dried blood at the LIENSs (La Rochelle, France). Aliquots of  $\sim 0.3$  mg (for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) and  $\sim 0.8$  mg (for  $\delta^{34}\text{S}$ ) dry mass were analyzed with a continuous flow mass spectrometer (Thermo Scientific Delta V Advantage) coupled to an elemental analyzer (Thermo Scientific Flash EA 1112). Results are presented as standard delta ( $\delta$ ) notation as parts per mil (‰) deviation relative to Vienna Pee Dee Belemnite, atmospheric  $\text{N}_2$  and Vienna Canyon Diablo Troilite for S) for  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and  $\delta^{34}\text{S}$ , respectively. Internal laboratory standards USGS-61 (Caffeine) and USGS-62 (Caffeine) for both C and N, and USGS-42 (human hair) and IAEA-S2 (silver sulfide) for S were used to check accuracy. Measurement errors were  $< 0.15\%$  for both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values, and  $< 0.20\%$  for  $\delta^{34}\text{S}$  values.

#### 2.5. Statistical analyses

The effects of the distance to the shore on variables were tested across the whole spatial scale of our study, and also within the closest area ( $<200$  m) to investigate localized effects at small scale (see results).

To test for the effect of distance to the ocean on blood isotopic values, simple linear models (lms) were computed, and we further tested if

individual traits (sex and body size) were significant covariates by using lms with size, sex and their interaction with distance to the ocean. A backward model selection using F tests (Bolker et al., 2009; Zuur et al., 2009) was carried out, and only the retained variables are represented (e.g. sex was never a significant predictor). A dataset of plasma osmolality values (Lorrain-Soligon et al., 2022) was also re-analyzed, with a focus on small scale localized effects within the sites situated close to the seashore. The relationships between isotopic values were analyzed, as were those between isotopic values and osmolality, using lms with osmolality or isotopic values as a covariate.

Finally, categories of distance to the seashore (close [ $<200$  m], intermediate [between 200 and 600 m], and distant [ $>600$  m]) were used to investigate the trophic niches of populations living closer to or further from the shore using Bayesian statistics with the Package SIBER: Stable Isotope Bayesian Ellipses in R (Jackson et al., 2011). These Bayesian models can be applied for populations if  $n > 10$  (Jackson et al., 2011) as it is the case in our populations. This approach computes the total area of the convex hull encompassing the data points (TA), representing the total extent of trophic diversity and niche width (Jackson et al., 2011; Layman et al., 2007), and the Standard Ellipse Area (SEA) or the corrected Standard Ellipse Area (SEAc) (Jackson et al., 2011), allowing to compare subpopulations. Overlap of  $\text{SEAc}$  among areas (close, intermediate, distant) was calculated for each population. Additionally, we compared populations using the range of  $\delta^{15}\text{N}$  values illustrating trophic diversity, the range of  $\delta^{13}\text{C}$  and  $\delta^{34}\text{S}$  values illustrating the diversity of basal resources, the mean distance to centroid (CCD) providing information on niche width as well as individual spacing, the mean nearest neighbour distance (MNND) illustrating density of individuals in the populations, and the standard deviation of the nearest neighbour distance (SDNND), a measure of evenness of spatial density and packing (Jackson et al., 2011; Layman et al., 2007).

All data analysis were performed using R 3.6.3 (R Core Team, 2020) and Rstudio v1.1.419.

### 3. Results

#### 3.1. Variations with distance to the ocean

In MO (« Réserve Naturelle Nationale de Moëze-Oléron ») when the whole spatial scale (close, intermediate and distant area) was considered, blood  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and  $\delta^{34}\text{S}$  values, as well as plasma osmolality, decreased with distance from the ocean (Table 1; Fig. 2 ACEG). This decrease was sharper for  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and osmolality when considering only the closest area (<200 m from the ocean, Table 1, Fig. 2 BDH), but this was not the case for  $\delta^{34}\text{S}$  for which, in the closest area, the isotopic value was not influenced by distance to the ocean (Table 1, Fig. 2F).

In MY (« Réserve Naturelle Nationale du marais d'Yves »), when the whole spatial scale was considered, plasma osmolality decreased significantly when distance to the ocean increased (Table 1, Fig. 2G). Blood  $\delta^{34}\text{S}$  values increased with distance from the ocean (Table 1, Fig. 2E), while  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  did not vary according to the distance to the ocean (Fig. 2 AC). Distance to coastline did not influence  $\delta^{15}\text{N}$ ,  $\delta^{13}\text{C}$ ,  $\delta^{34}\text{S}$  or osmolality on the small spatial scale (i.e. on the closest area only, <200 m from the ocean, Fig. 2 BDFH).

#### 3.2. Effects of individual traits

In interaction with distance to the coast, a negative effect of body size on  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values was found only in MO, and when considering the closest area only (<200 m, Table 1, Appendix B). For all other isotopic values and osmolality, and for all sites and areas, there were no effects of size, sex, and of their interaction with distance to the coast (Table 1).

#### 3.3. Correlations between isotopic values

In MO, across the whole spatial scale,  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values were correlated ( $R^2 = 0.615$ ,  $p\text{-value} < 0.001$ ), as well as  $\delta^{15}\text{N}$  and  $\delta^{34}\text{S}$  values ( $R^2 = 0.140$ ,  $p\text{-value} = 0.015$ ), but not  $\delta^{13}\text{C}$  and  $\delta^{34}\text{S}$  ( $R^2 = 0.006$ ,  $p\text{-value} = 0.279$ ). When restricting our analyses to the closest area (<200 m from the ocean),  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values were correlated ( $R^2 = 0.986$ ,  $p\text{-value} < 0.001$ ), but no correlation with  $\delta^{34}\text{S}$  values was significant (all  $R^2 < -0.064$ , all  $p\text{-value} > 0.545$ ).

In MY across the whole spatial scale, none of the isotopic values was correlated (all  $R^2 < 0.057$ , all  $p\text{-value} > 0.100$ ). When restricting our analyses to the closest area (<200 m from the ocean),  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values were correlated ( $R^2 = 0.617$ ,  $p\text{-value} = 0.001$ ), but no correlation with  $\delta^{34}\text{S}$  values was significant (all  $R^2 < -0.064$ , all  $p\text{-value} > 0.545$ ).

#### 3.4. Correlations between isotopic values and osmolality

In MO, both across the whole spatial scale and when restricting our analyses to the closest area (<200 m from the ocean), blood  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values were correlated with plasma osmolality (all  $R^2 > 0.607$ , all  $p\text{-value} < 0.001$ , Fig. 3 ABCD), but this was not the case for  $\delta^{34}\text{S}$  values (whole spatial scale,  $R^2 = -0.011$ ,  $p\text{-value} = 0.428$ , Fig. 3E; closest area,  $R^2 = -0.085$ ,  $p\text{-value} = 0.655$ , Fig. 3F).

In MY,  $\delta^{15}\text{N}$ ,  $\delta^{13}\text{C}$  and  $\delta^{34}\text{S}$  values were not correlated to osmolality (all  $R^2 < 0.191$ , all  $p\text{-value} > 0.087$ , Fig. 3 ABCDEF).

#### 3.5. Comparisons of isotopic niches

Western spadefoot toads from the two sites differed in their isotopic niche. In particular the  $\delta^{13}\text{C}$ - $\delta^{15}\text{N}$  niche (TA) was larger in MO compared to MY, with wider  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  ranges, more dispersion in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values, more trophic diversity (CCD) and trophic evenness (SDNND) for the  $\delta^{13}\text{C}$ - $\delta^{15}\text{N}$  range (Table 2). By contrast, the  $\delta^{13}\text{C}$ - $\delta^{34}\text{S}$  niche (TA) was larger in MY compared to MO, with a wider  $\delta^{34}\text{S}$  range, and more dispersion in  $\delta^{34}\text{S}$  values (Table 2). Individuals from MY also expressed more trophic diversity (CCD) and trophic evenness (SDNND) for the  $\delta^{13}\text{C}$ - $\delta^{34}\text{S}$  range (Table 2).

When considering isotopic niches with  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values, we found that the trophic niche (TA and SEA) was larger closer to the ocean (in the closest area, <200 m from the ocean) in both sites (Fig. 4 AB, Table 3).

Concerning isotopic niches with  $\delta^{13}\text{C}$  and  $\delta^{34}\text{S}$  values, we also found that the trophic niches (TA and SEA) were larger closer to the ocean (in the closest area, <200 m from the ocean) in both sites (Fig. 4 CD, Table 3).

Overlaps between the close, intermediate and distant areas were smaller in MO than in MY (Appendix C).

### 4. Discussion

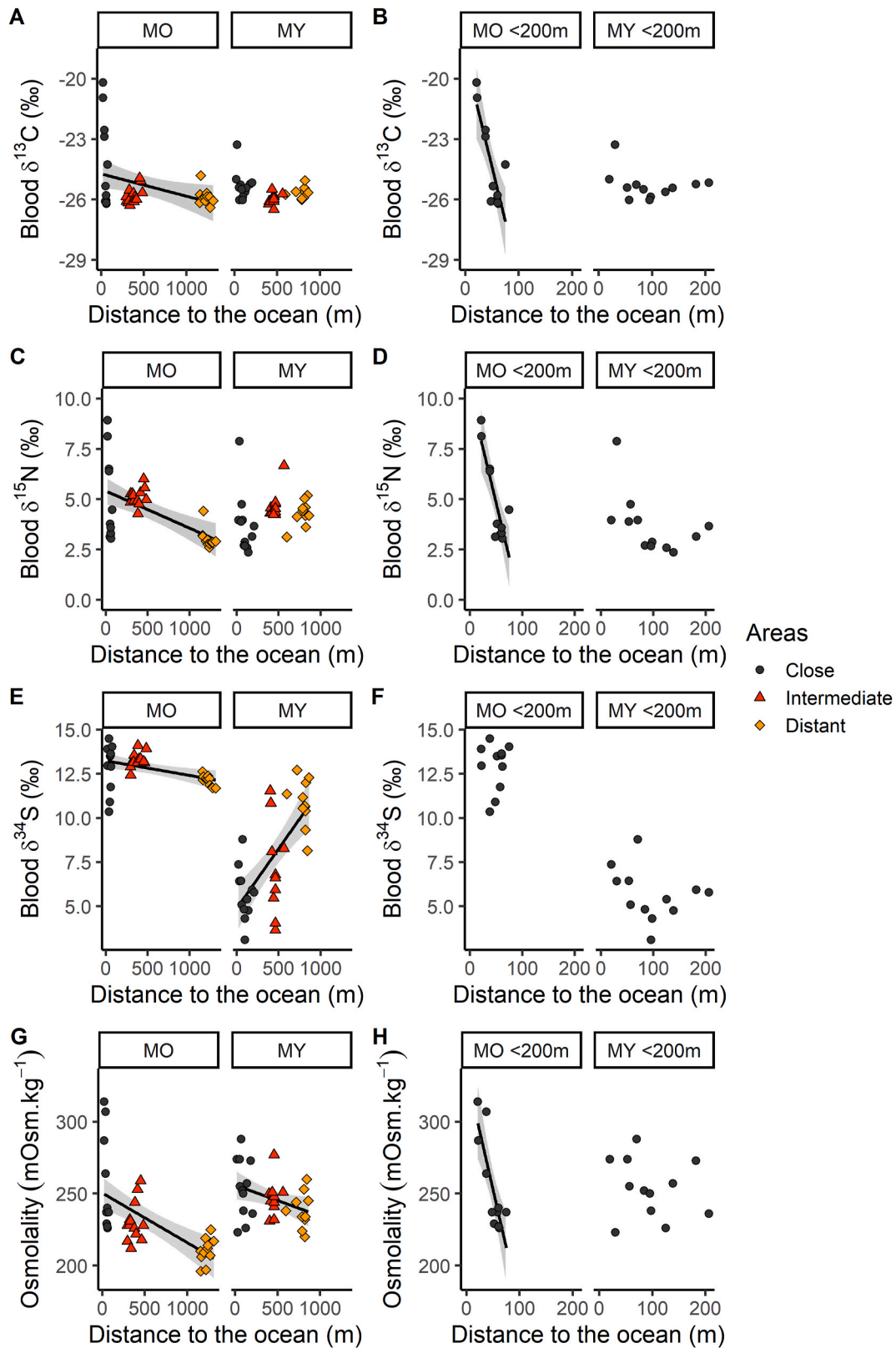
In this study, we tested whether blood stable isotope values are useful to assess habitat use (distance to the shoreline) across terrestrial coastal wetlands in an amphibian, the western spadefoot toad *Pelobates cultripes*. Because coastal winds cause landward transport of matter, marine influence was expected to progressively decrease with increasing distance from the ocean (Meira et al., 2008; Mustafa and Yusof, 1994). Overall, our results highlighted divergent site-specific responses (Denton et al., 2019). In one of our study sites (MO), as predicted, all three elements investigated (blood  $\delta^{15}\text{N}$ ,  $\delta^{13}\text{C}$  and  $\delta^{34}\text{S}$  values) decreased with increasing distance from the ocean. However, in the other study site (MY), two of the isotopic values ( $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ ) did not vary with distance to the shoreline and  $\delta^{34}\text{S}$  values showed a trend opposite to our

**Table 1**

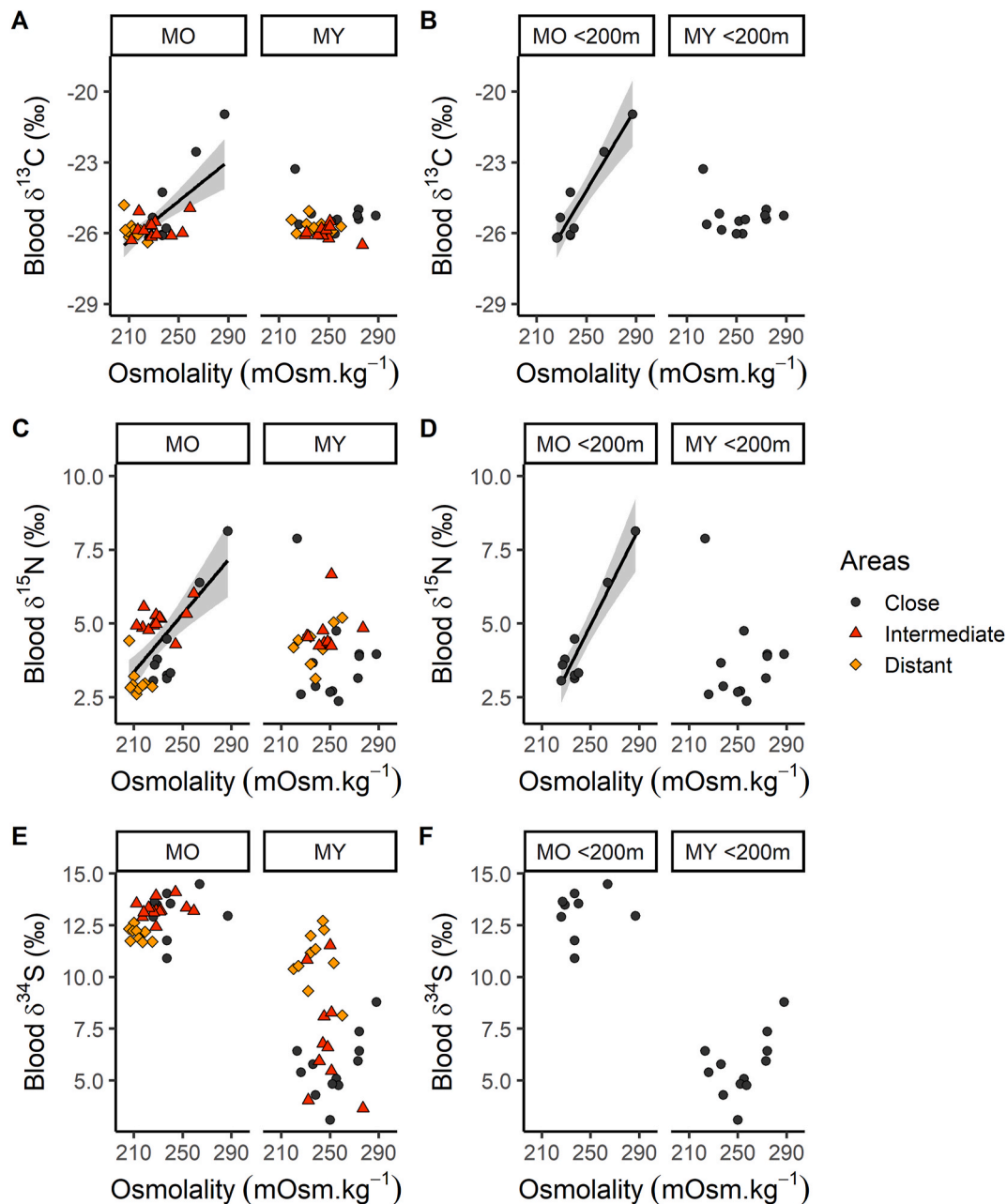
Effects of distance to the ocean, size and their interaction on blood  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and  $\delta^{34}\text{S}$  values, and plasma osmolality in MY and MO across the whole study sites (close, intermediate, distant), or restricted to the closest area. MO: Réserve Naturelle Nationale de Moëze-Oléron and MY: Réserve Naturelle Nationale du marais d'Yves. Note that only the retained covariates are represented.

	Sites	Covariates	MO					MY				
			$R^2$	Estimate	SE	t	p	$R^2$	Estimate	SE	t	p
Blood $\delta^{13}\text{C}$	All areas	Distance	0.114	-0.001	<0.001	-2.314	0.027	0.057	<-0.001	<0.001	-1.693	0.101
	Closest area only	Distance	0.901	0.325	0.125	2.607	0.035	-0.028	-0.003	0.004	-0.838	0.422
		Size		0.246	0.097	2.551	0.038		-	-	-	-
		Distance*Size		-0.007	0.002	-3.554	0.009		-	-	-	-
Blood $\delta^{15}\text{N}$	All areas	Distance	0.324	-0.002	<0.001	-4.156	<0.001	0.030	0.001	0.001	1.404	0.171
	Closest area only	Distance	0.924	0.264	0.105	2.504	0.041	0.179	-0.013	0.007	-1.846	0.095
		Size		0.205	0.082	2.512	0.04		-	-	-	-
		Distance*Size		-0.006	0.002	-3.614	0.009		-	-	-	-
Blood $\delta^{34}\text{S}$	All areas	Distance	0.167	-0.001	<0.001	-2.791	0.009	0.467	0.007	0.001	5.31	<0.001
	Closest area only	Distance	-0.1	0.007	0.026	0.265	0.797	-0.042	-0.007	0.008	-0.946	0.367
Plasma Osmolality	All areas	Distance	0.375	-0.035	0.007	-4.623	<0.001	0.114	-0.022	0.01	-2.235	0.033
	Closest area only	Distance	0.676	-1.614	0.345	-4.677	0.001	-0.066	-0.063	0.111	-0.567	0.584





**Fig. 2.** Blood  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ,  $\delta^{34}\text{S}$  values, and plasma osmolality in relation to distance to the ocean across the whole study sites (left column) and restricted to the closest area (right column). Regression lines are used for significant relationships solely. MO: Réserve Naturelle Nationale de Moëze-Oléron and MY: Réserve Naturelle Nationale du marais d'Yves. Panel G is redrawn from Lorrain-Soligon et al. (2022).



**Fig. 3.** Correlations between blood  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ,  $\delta^{34}\text{S}$  values, and plasma osmolality across the whole study sites (left column) and restricted to the closest area (right column). Regression lines are used for significant relationships solely. MO: Réserve Naturelle Nationale de Moëze-Oléron and MY: Réserve Naturelle Nationale du marais d'Yves.

prediction. Nevertheless, individual osmolality (related to landward transport of sea-spray and salt intake, Lorrain-Soligon et al., 2022) showed a decrease according to distance to the ocean at both sites.

In MO, we found that all three isotopic values decreased with increasing distance from the shoreline as expected from a progressive decline in marine matter deposition with increasing distance from the ocean. This trend was also detectable at a smaller spatial scale (<200 m from the coastline) for both  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values. Such a pattern suggests that the deposited marine matter is integrated within the trophic web, from primary producers up to meso-predators such as the western spadefoot toad. All three isotopic values were related to one another when considering the whole spatial scale, which strengthens this hypothesis. The strong effect close to the shoreline (see slopes in Fig. 2AC versus Fig. 2BD, Table 1) suggests that this process occurs primarily at a very short spatial scale, as expected from the spatial extent of the

windward transport of relatively heavy particles such as sediment and organic matter (Bainbridge et al., 2018; Ridderinkhof, 1998). Interestingly,  $\delta^{34}\text{S}$  variation showed a similar trend across the whole spatial scale of our study, but no significant trend when restricting our analyses to the close study area (<200 m). These results suggest that  $\delta^{34}\text{S}$  values does not vary on such a restricted spatial scale, as shown in other studies (Nehlich, 2015; Novák et al., 2001). Blood  $\delta^{34}\text{S}$  values could thus decrease on a larger spatial scale, only a small part of which being covered by our sampling (Nehlich, 2015; Novák et al., 2001). In line with these results, we found that individual osmolality (related to salt intake and linked to landward transport of sea-spray, Lorrain-Soligon et al., 2022) dovetails remarkably well with the isotopic trends. Plasma osmolality was strongly linked to both blood  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values either at the global scale, or when restricted to the closest study area. It is noteworthy that both sets of markers provide information at different

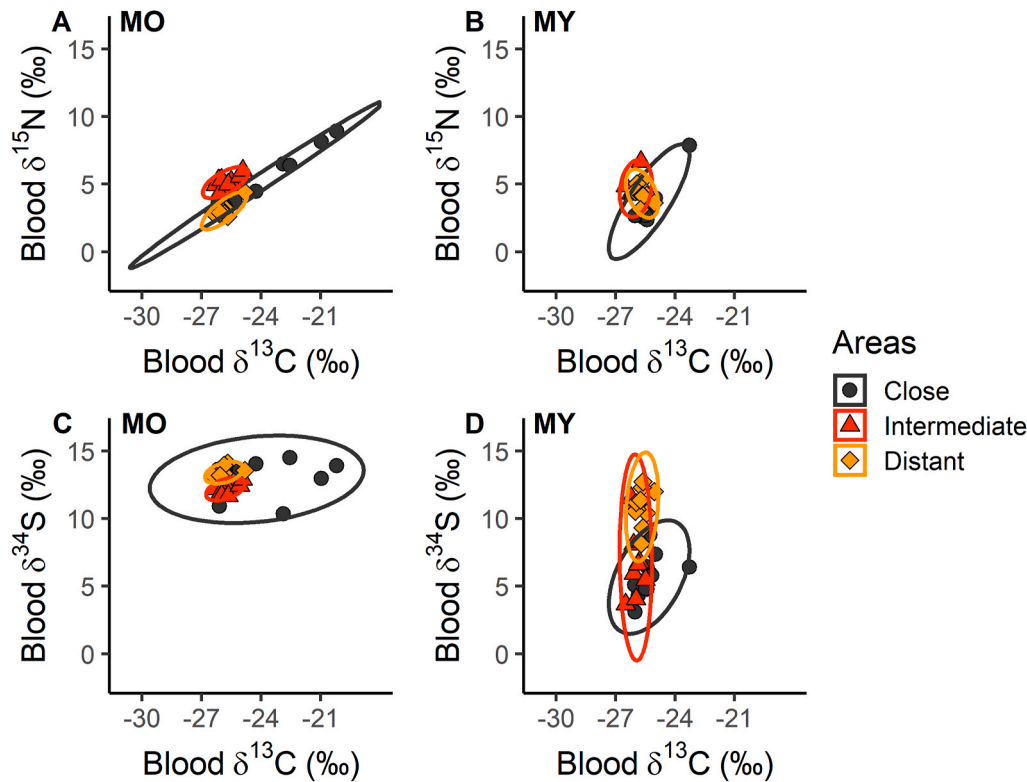
**Table 2**

Isotopic site metrics for blood  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values (upper line), and blood  $\delta^{13}\text{C}$  and  $\delta^{34}\text{S}$  values (lower line), computed from SIBER for MO (Réserve Naturelle Nationale de Moëze-Oléron) and MY (Réserve Naturelle Nationale du marais d'Yves):  $\delta^{13}\text{C}$  ( $\delta^{13}\text{C}$  range),  $\delta^{15}\text{N}$  ( $\delta^{15}\text{N}$  range) and  $\delta^{34}\text{S}$  ( $\delta^{34}\text{S}$  range), Total Area of the convex hull encompassing the data points (TA), mean Distance to Centroid (CCD), Mean Nearest Neighbour Distance (MNND), and Standard Deviation of the Nearest Neighbour Distance (SDNND).

		MO	MY
Blood $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$	$\delta^{15}\text{N}$ range	2.086	0.990
	$\delta^{13}\text{C}$ range	1.632	0.666
	TA	1.640	0.028
	CCD	1.190	0.423
	MNND	1.746	0.564
	SDNND	0.295	0.119
Blood $\delta^{13}\text{C}$ and $\delta^{34}\text{S}$	$\delta^{34}\text{S}$ range	1.205	5.182
	$\delta^{13}\text{C}$ range	1.632	0.666
	TA	0.967	1.472
	CCD	0.894	2.013
	MNND	1.372	2.311
	SDNND	0.286	1.246

temporal scale. Blood isotopic values provide information according to the life of erythrocytes that lasts several months in amphibians (Altland and Brace, 1962; Cloyed et al., 2015). Plasma osmolality is much more dynamic (e.g., days or weeks) and varies according to freshwater intake (e.g., during rainfall) that allows equilibrating an individual's hydric state (Hillyard et al., 1998; Park and Do, 2020). Such similar trends for markers that integrate different temporal scales suggest individual specialization in habitat use. Future studies combining mark-recapture studies and repeated measures of isotopic values (on plasma and red blood cells, see Denton et al., 2019) and plasma osmolality should usefully test for this hypothesis.

In strong contrast with the results from MO, none of the isotopic values displayed the expected trends at the MY site. Neither  $\delta^{15}\text{N}$  nor  $\delta^{13}\text{C}$  values were related to distance to the shoreline, either at the whole spatial scale or when restricting our analyses to the closest area solely. This divergence was even stronger for  $\delta^{34}\text{S}$  values, which increased with increasing distance from the shoreline. Similarly to MO, osmolality of individuals from MY decreased with increasing distance from the ocean (Lorrain-Soligon et al., 2022), but, unlike MO, osmolality was not related to any isotopic value. Although such contrast between close and



**Fig. 4.** Blood isotopic niches for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  (upper panels) and  $\delta^{13}\text{C}$  and  $\delta^{34}\text{S}$  (lower panels) in MO (left column) and MY (right column) as determined using SIBER. MO: Réserve Naturelle Nationale de Moëze-Oléron and MY: Réserve Naturelle Nationale du marais d'Yves.

**Table 3**

Subpopulations metrics computed from SIBER for blood  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values (upper line), and blood  $\delta^{13}\text{C}$  and  $\delta^{34}\text{S}$  values (lower line). Total Area of the convex hull encompassing the data points (TA), Standard Ellipse Area (SEA) and corrected Standard Ellipse Area (SEAc) in the close, intermediate and distant areas in MO and MY. MO: Réserve Naturelle Nationale de Moëze-Oléron and MY: Réserve Naturelle Nationale du marais d'Yves.

		MO			MY		
	Areas	Close	Intermediate	Distant	Close	Intermediate	Distant
Blood $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$	TA	2.961	0.855	0.847	4.863	1.350	1.062
	SEA	1.693	0.416	0.386	2.010	0.627	0.491
	SEAc	1.881	0.454	0.428	2.210	0.705	0.552
Blood $\delta^{13}\text{C}$ and $\delta^{34}\text{S}$	TA	16.788	0.980	0.765	7.323	4.338	2.196
	SEA	9.195	0.421	0.390	2.978	2.307	1.195
	SEAc	10.216	0.460	0.433	3.276	2.595	1.345

structurally similar study sites can appear puzzling, we believe the underlying reason is related to the recent history of these sites. Indeed, both sites were hit by storm Xynthia in 2010, but the spatial extent of the associated marine submersion was strongly different between the two sites, due to their slightly different topography (i.e., additional sand dune in MO compared to MY; Lorrain-Soligon et al., 2021). MY was entirely submerged and seawater have been retained several weeks, especially in lowland areas. The resulting salinization was still detectable seven years after the submersion (Lorrain-Soligon et al., 2021). By contrast, owing to its topography (Lorrain-Soligon et al., 2021), coastal wetlands of MO were spared from the marine submersion induced by storm Xynthia (Lorrain-Soligon et al., 2021). This suggests that the effects of a marine submersion and the resulting deposition of marine elements (salt, sediments, and organic matter) can disrupt isotopic signatures of coastal areas, a process that appears to be long-lasting as our isotopic sampling occurred 11 years post-submersion. If such hypothesis holds true, it is a major element to take into account when integrating isotopic analyses to habitat use and individual movements in coastal ecosystems, as it seems necessary to take into account the history of marine intrusions to understand the spatio-temporal dynamics of isotopic values (Baumann et al., 2017; Raji et al., 2015). However, this hypothesis cannot fully explain the  $\delta^{34}\text{S}$  variation at MY, as well as the strong contrast of  $\delta^{34}\text{S}$  values close to seashore between MO and MY. Such contrasts between close and structurally similar study sites remain puzzling and clearly deserve future investigations. Overall,  $\delta^{34}\text{S}$  isoscapes are known to be highly variable (Nehlich, 2015), and such variation may explain the trends we found. Future studies are required to decipher the bases for such divergences between close and structurally similar coastal wetlands. Extending our investigations to other coastal taxa may also provide critical insights on the variation of  $\delta^{34}\text{S}$  values.

As a consequence of these divergent spatial dynamics of isotopic values, trophic niches of western spadefoot toads were also different between our study sites. Blood  $\delta^{34}\text{S}$  range was higher in MY, suggesting more marine-derived nutrients in the habitat (Hesslein et al., 1991; MacAvoy et al., 2000). However,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  ranges were higher in MO, suggesting wider food resources, as these elements reflect the variability of basal food resources (Potapov et al., 2019). Interestingly, in both study sites, we found a larger isotopic niche closer to the seashore, both considering the  $\delta^{13}\text{C}$ - $\delta^{15}\text{N}$  or  $\delta^{13}\text{C}$ - $\delta^{34}\text{S}$  niches. Such results suggests that, at both sites, individuals that live close to the seashore have access to a wider spectrum of food resources (Carmo et al., 2021). Indeed, anecdotal observations of prey regurgitation during sampling showed that individuals captured further from the seashore feed on insects (rove beetles) and terrestrial gastropods (slugs and snails), while individuals captured near the seashore incorporate marine prey (sand hoppers) as well as terrestrial prey in their diet, indicating trophic adaptability (Andolina et al., 2022), or an opportunistic feeding behavior (Shaiek et al., 2015). Additionally, the larger isotopic niche close to the seashore could be due to a greater range in isotopic values of the potential preys, and thus more variation at the base of the food web. Measurements of isotope values of preys are required to test for these hypotheses. Despite divergence in overall isotopic values, such dietary variation according to the distance to the ocean seems similar between the two study sites.

## 5. Conclusions

Taken together these results suggest that blood  $\delta^{15}\text{N}$ ,  $\delta^{13}\text{C}$  and  $\delta^{34}\text{S}$  values can provide insight on habitat use, but their usefulness is site-specific. Indeed, one needs to be cautious with the recent history (e.g., marine submersion) of a site before applying this technique. Moreover,  $\delta^{34}\text{S}$  values may be suitable to discriminate marine habitats from terrestrial ones, but less useful to infer habitat use at a smaller spatial scale, such as those characterizing coastal wetlands. As such, we recommend using  $\delta^{34}\text{S}$  values as a tool to understand habitat use in

coastal environments, assuming that history of recent marine intrusions, topological profiles of the sites, and land cover are well known. Indeed, coastal habitats are complex environmental matrix with multiple influences, resulting in many different types of habitats spread over a restricted spatial scale. Combining the stable isotope method with other habitat use metrics (e.g., osmolality in the current study) seems to be relevant to comprehensively understand isoscapes in a site-specific context.

## Ethics statement

This work was approved by the French authorities under permits R-45GRETA-F1-10 and 135-2020 DBEC.

## Funding statement

Funding was provided by the CNRS, La Rochelle Université, the LPO, the Agence de l'Eau Adour-Garonne, the Conseil Départemental de la Charente-Maritime, the ANR PAMPAS (ANR-18-CE32-0006), the Contrat de plan Etat-Région Econat and the Fonds Européen de Développement Régional (FEDER). Funders had no role in study design; in the collection, analysis and interpretation of data; in the writing of the report; nor in the decision to submit the article for publication.

## CRediT authorship contribution statement

**Léa Lorrain-Soligon:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Frédéric Robin:** Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Vincent Lelong:** Writing – review & editing, Validation, Resources, Methodology, Investigation, Data curation. **Marko Jankovic:** Writing – review & editing, Validation, Project administration, Methodology, Investigation, Data curation. **Paco Bustamante:** Writing – review & editing, Writing – original draft, Validation, Methodology, Formal analysis. **Yves Cherel:** Writing – review & editing, Writing – original draft, Validation, Methodology, Formal analysis. **François Brischoux:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

## Acknowledgements

The authors would like to thank Philippe Delaporte, Pierre Rousseau, Loïc Jomat, Nathalie Bourret, Julia Guerra Carande, Flavie Rouet, Guillaume Seglen, Bernard Queval, Christophe Dufour, Claude Champarnaud, Thomas Herault, Karine Vennel, Ludovic Pépin, Camille Pereira, Nathan Chouteau, François Villain, Yakov Uzan and all volunteers for their help during field work. We are also thankful to Gaël Guillou from the “Plateforme analyses isotopiques” of LIENSs (La Rochelle Université) for computing isotopic analyses. Thanks are due to the Contrat de Projet Etat-Région (CPER) and the Fonds Européen de Développement Régional (FEDER) for funding the isotope-ratio mass spectrometers of LIENSs laboratory. The Institut Universitaire de France



(IUF) is acknowledged for its support to Paco Bustamante as a Senior Member.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ecss.2022.107953>.

## References

- Altland, P.D., Brace, K.C., 1962. Red cell life span in the turtle and toad. *Am. J. Physiol.-Leg. Content* 203, 1188–1190. <https://doi.org/10.1152/ajplegacy.1962.203.6.1188>.
- Andolina, C., Franzoi, P., Cavraro, F., Jackson, A.L., Mazzola, A., Vizzini, S., 2022. Trophic adaptability shapes isotopic niche of the resident fish *Aphanius fasciatus* across lagoon habitats. *Estuar. Coast Shelf Sci.* 264, 107685 <https://doi.org/10.1016/j.ecss.2021.107685>.
- Arthur, K., Boyle, M., Limpus, C., 2008. Ontogenetic changes in diet and habitat use in green sea turtle (*Chelonia mydas*) life history. *Mar. Ecol. Prog. Ser.* 362, 303–311. <https://doi.org/10.1016/j.marpolbul.2018.08.002>.
- Bainbridge, Z., Lewis, S., Bartley, R., Fabricius, K., Collier, C., Waterhouse, J., Garzon-Garcia, A., Robson, B., Burton, J., Wenger, A., Brodie, J., 2018. Fine sediment and particulate organic matter: a review and case study on ridge-to-reef transport, transformations, fates, and impacts on marine ecosystems. *Mar. Pollut. Bull.* 135, 1205–1220. <https://doi.org/10.1016/j.marpolbul.2018.08.002>.
- Barbier, E.B., 2015. Valuing the storm protection service of estuarine and coastal ecosystems. *Ecosyst. Serv., Marine Eco Pol Related Ecosys. Serv.: Lessons from the World's Regional Seas* 11, 32–38. <https://doi.org/10.1016/j.ecoser.2014.06.010>.
- Baumann, J., Chaumillon, E., Schneider, J.-L., Jorissen, F., Sauriau, P.-G., Richard, P., Bonnin, J., Schmidt, S., 2017. Contrasting sediment records of marine submersion events related to wave exposure. *Southwest France. Sediment. Geol.* 353, 158–170. <https://doi.org/10.1016/j.sedgeo.2017.03.009>.
- Bearhop, S., Furness, R.W., Hilton, G.M., Votier, S.C., Waldron, S., 2003. A forensic approach to understanding diet and habitat use from stable isotope analysis of (avian) claw material. *Funct. Ecol.* 17, 270–275. <https://doi.org/10.2307/3599183>.
- Benassai, S., Becagli, S., Gragnani, R., Magand, O., Proposito, M., Fattori, I., Traversi, R., Udisti, R., 2005. Sea-spray deposition in Antarctic coastal and plateau areas from ITASE traverses. *Ann. Glaciol.* 41, 32–40. <https://doi.org/10.3189/172756405781813285>.
- Bolker, B.M., Brooks, M.E., Clark, C.J., Geange, S.W., Poulsen, J.R., Stevens, M.H.H., White, J.-S.S., 2009. Generalized linear mixed models: a practical guide for ecology and evolution. *Trends Ecol. Evol.* 24, 127–135. <https://doi.org/10.1016/j.tree.2008.10.008>.
- Brischoux, F., Tingley, R., Shine, R., Lillywhite, H.B., 2013. Behavioral and physiological correlates of the geographic distributions of amphibious sea kraits (*Laticauda* spp.). *J. Sea Res.* 76, 1–4. <https://doi.org/10.1016/j.seares.2012.10.010>.
- Brüssow, H., Parkinson, S.J., 2014. You are what you eat. *Nat. Biotechnol.* 32, 243–245. <https://doi.org/10.1038/nbt.2845>.
- Carmo, L.F., Alves, A.A., Woitovitz-Cardoso, M., Sabagh, L.T., 2021. Eat on the beach: diet composition of an endemic miniature frog from sandy coastal environments of southeastern Brazil. *Stud. Neotrop. Fauna Environ.* 1–10. <https://doi.org/10.1080/01650521.2021.1877414>.
- Choy, E.J., Park, H., Kim, J.-H., Ahn, I.-Y., Kang, C.-K., 2011. Isotopic shift for defining habitat exploitation by the Antarctic limpet *Nacella concinna* from rocky coastal habitats (Marian Cove, King George Island). *Estuar. Coast Shelf Sci.* 92, 339–346. <https://doi.org/10.1016/j.ecss.2011.01.009>.
- Cloyd, C.S., Newsome, S.D., Eason, P.K., 2015. Trophic discrimination factors and incorporation rates of carbon and nitrogen-stable isotopes in adult green frogs. *Lithobates clamitans*. *Physiol. Biochem. Zool.* 88, 576–585. <https://doi.org/10.1086/682576>.
- Core Team, R., 2020. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Austria: Vienna. <https://www.R-project.org/>.
- Demoisson, A., Tedeschi, G., Piazzola, J., 2013. A model for the atmospheric transport of sea-salt particles in coastal areas. *Atmos. Res.* 132–133, 144–153. <https://doi.org/10.1016/j.atmosres.2013.04.002>.
- Denton, M.J., Demopoulos, A.W.J., Baldwin, J.D., Smith, B.J., Hart, K.M., 2019. Stable isotope analysis enhances our understanding of diamondback terrapin (*Malaclemys terrapin*) foraging ecology. *Estuar. Coast* 42, 596–611. <https://doi.org/10.1007/s12237-018-0476-6>.
- Dettinger, M., 2011. Climate change, atmospheric rivers, and floods in California - a multimodel analysis of storm frequency and magnitude changes. *JAWRA J. Am. Water Resour. Assoc.* 47, 514–523. <https://doi.org/10.1111/j.1752-1688.2011.00546.x>.
- Diehl, K.-H., Hull, R., Morton, D., Pfister, R., Rabemampianina, Y., Smith, D., Vidal, J.-M., Vorstenbosch, C.V.D., 2001. A good practice guide to the administration of substances and removal of blood, including routes and volumes. *J. Appl. Toxicol.* 21, 15–23. <https://doi.org/10.1002/jat.727>.
- Fry, B., 2002. Conservative mixing of stable isotopes across estuarine salinity gradients: a conceptual framework for monitoring watershed influences on downstream fisheries production. *Estuaries* 25, 264–271. <https://doi.org/10.1007/BF02691313>.
- Fry, B., Chumchal, M.M., 2011. Sulfur stable isotope indicators of residency in estuarine fish. *Limnol. Oceanogr.* 56, 1563–1576. <https://doi.org/10.4319/lo.2011.56.5.1563>.
- Hesslein, R.H., Capel, M.J., Fox, D.E., Hallard, K.A., 1991. Stable isotopes of sulfur, carbon, and nitrogen as indicators of trophic level and fish migration in the lower mackenzie river basin, Canada. *Can. J. Fish. Aquat. Sci.* 48, 2258–2265. <https://doi.org/10.1139/f91-265>.
- Hillyard, S.D., Hoff, K. von S., Propper, C., 1998. The water absorption response: a behavioral assay for physiological processes in terrestrial amphibians. *Physiol. Zool.* 71, 127–138. <https://doi.org/10.1086/515900>.
- Hobohm, C., Schaminée, J., van Rooijen, N., 2021. Coastal habitats, shallow seas and inland saline steppes: ecology, distribution, threats and challenges. In: Hobohm, C. (Ed.), *Perspectives for Biodiversity and Ecosystems, Environmental Challenges and Solutions*. Springer International Publishing, Cham, pp. 279–310. [https://doi.org/10.1007/978-3-030-57710-0\\_12](https://doi.org/10.1007/978-3-030-57710-0_12).
- Hussain, M.I., Al-Dakheel, A.J., 2018. Effect of salinity stress on phenotypic plasticity, yield stability, and signature of stable isotopes of carbon and nitrogen in safflower. *Environ. Sci. Pollut. Res.* 25, 23685–23694. <https://doi.org/10.1007/s11356-018-2442-z>.
- Jackson, A.L., Inger, R., Parnell, A.C., Bearhop, S., 2011. Comparing isotopic niche widths among and within communities: SIBER – stable Isotope Bayesian Ellipses in R. *J. Ecol.* 80, 595–602. <https://doi.org/10.1111/j.1365-2656.2011.01806.x>.
- Kohn, M.J., 1999. You are what you eat. *Science* 283, 335–336. <https://doi.org/10.1126/science.283.5400.335>.
- Kudman, S., 2021. A Comparison of the Diets of Three Diamondback Terrapin Populations Using Fecal Analysis and Stable Isotope Analysis (Doctoral Dissertation, Hofstra University) (PhD Thesis). Hofstra University.
- Kulp, S.A., Strauss, B.H., 2019. New elevation data triple estimates of global vulnerability to sea-level rise and coastal flooding. *Nat. Commun.* 10, 1–12. <https://doi.org/10.1038/s41467-019-12808-z>.
- Layman, C.A., Arrington, D.A., Montaña, C.G., Post, D.M., 2007. Can stable isotope ratios provide for community-wide measures of trophic structure? *Ecology* 88, 42–48. [https://doi.org/10.1890/0012-9658.2007.88\[42:CSIRPF\]2.0.CO;2](https://doi.org/10.1890/0012-9658.2007.88[42:CSIRPF]2.0.CO;2).
- Layman, C.A., Araujo, M.S., Boucek, R., Hammerschlag-Peyer, C.M., Harrison, E., Jud, Z. R., Matich, P., Rosenblatt, A.E., Vaudo, J.J., Yeager, L.A., Post, D.M., Bearhop, S., 2012. Applying stable isotopes to examine food-web structure: an overview of analytical tools. *Biol. Rev.* 87, 545–562. <https://doi.org/10.1111/j.1469-185X.2011.00208.x>.
- Leclair, M.H., Leclair, R., Gallant, J., 2005. Application of skeletochronology to a population of *Pelobates cultripes* (Anura: pelobatidae) from Portugal. *J. Herpetol.* 39, 199–207. <https://doi.org/10.1670/174-044>.
- Leyden, E., Farkas, J., Gilbert, S., Hutson, J., Mosley, L.M., 2021. A simple and rapid ICP-MS/MS determination of sulfur isotope ratios (34S/32S) in complex natural waters: a new tool for tracing seawater intrusion in coastal systems. *Talanta* 235, 122708. <https://doi.org/10.1016/j.talanta.2021.122708>.
- Lizana, M., Márquez, R., Martín-Sánchez, R., 1994. Reproductive biology of *Pelobates cultripes* in Central Spain. *J. Herpetol.* 28, 19–27. <https://doi.org/10.2307/1564675>.
- Lorrain-Soligon, L., Robin, F., Rousseau, P., Jankovic, M., Brischoux, F., 2021. Slight variations in coastal topography mitigate the consequence of storm-induced marine submersion on amphibian communities. *Sci. Total Environ.* 770, 145382 <https://doi.org/10.1016/j.scitotenv.2021.145382>.
- Lorrain-Soligon, L., Robin, F., Lelong, V., Jankovic, M., Barbraud, C., Brischoux, F., 2022. Distance to coastline modulates morphology and population structure in a coastal amphibian. *Biol. J. Linn. Soc.* 135, 478–489. <https://doi.org/10.1093/biolinnean/blab165>.
- Lott, C., Meehan, T., Heath, J., 2003. Estimating the latitudinal origins of migratory birds using hydrogen and sulfur stable isotopes in feathers: influence of marine prey base. *Oecologia* 134, 505–510. <https://doi.org/10.1007/s00442-002-1153-8>.
- MacAvoy, S.E., Macko, S.A., McIninch, S.P., Garman, G.C., 2000. Marine nutrient contributions to freshwater apex predators. *Oecologia* 122, 568–573. <https://doi.org/10.1007/s0044200050980>.
- Maynard, L., Wilcox, D., 1997. Coastal wetlands. *Tech. Rep.*
- McCue, M.D., Javal, M., Clusella-Trullas, S., Le Roux, J.J., Jackson, M.C., Ellis, A.G., Richardson, D.M., Valentine, A.J., Terblanche, J.S., 2020. Using stable isotope analysis to answer fundamental questions in invasion ecology: progress and prospects. *Methods Ecol. Evol.* 11, 196–214. <https://doi.org/10.1111/2041-210X.13327>.
- McLean, R.F., Tsyban, A., Burkett, V., Codignotto, J.O., Forbes, D.L., Mimura, N., Beamish, R.J., Ittekkot, V., 2001. Coastal zones and marine ecosystems. *Clim. Change* 343–379.
- Meira, G.R., Andrade, C., Alonso, C., Padaratz, I.J., Borba, J.C., 2008. Modelling sea-salt transport and deposition in marine atmosphere zone – a tool for corrosion studies. *Corrosion Sci.* 50, 2724–2731. <https://doi.org/10.1016/j.corsci.2008.06.028>.
- Michener, R.M., 1994. Stable isotope ratios as tracers in marine aquatic food webs. *Stable Isot. Ecol. Environ. Sci.* 2, 138–186.
- Minagawa, M., Wada, E., 1984. Stepwise enrichment of 15N along food chains: further evidence and the relation between  $\delta^{15}N$  and animal age. *Geochim. Cosmochim. Acta* 48, 1135–1140. [https://doi.org/10.1016/0016-7037\(84\)90204-7](https://doi.org/10.1016/0016-7037(84)90204-7).
- Monti, F., Robert, A., Dominici, J.-M., Sforzi, A., Bagur, R.T., Navarro, A.M., Guillou, G., Duriez, O., Bentaleb, I., 2021. Using GPS tracking and stable multi-isotopes for estimating habitat use and winter range in Palearctic ospreys. *Oecologia* 195, 655–666. <https://doi.org/10.1007/s00442-021-04855-5>.
- Mustafa, M.A., Yusuf, K.M., 1994. Atmospheric chloride penetration into concrete in semitropical marine environment. *Cement Concr. Res.* 24, 661–670. [https://doi.org/10.1016/0008-8846\(94\)90190-2](https://doi.org/10.1016/0008-8846(94)90190-2).
- Nehlich, O., 2015. The application of sulfur isotope analyses in archaeological research: a review. *Earth Sci. Rev.* 142, 1–17. <https://doi.org/10.1016/j.earscirev.2014.12.002>.

- Novák, M., Bottrell, S.H., Echová, E.P., 2001. Sulfur isotope inventories of atmospheric deposition, spruce forest floor and living Sphagnum along a NW–SE transect across Europe. *Biogeochemistry* 53, 23–50. <https://doi.org/10.1023/A:1010792205756>.
- Park, J.-K., Do, Y., 2020. Physiological response of *Pelophylax nigromaculatus* adults to salinity exposure. *Animals* 10, 1698. <https://doi.org/10.3390/ani10091698>.
- Pelegrí, J.L., Aristegui, J., Cana, L., González-Dávila, M., Hernández-Guerra, A., Hernández-León, S., Marrero-Díaz, A., Montero, M.F., Sangrà, P., Santana-Casiano, M., 2005. Coupling between the open ocean and the coastal upwelling region off northwest Africa: water recirculation and offshore pumping of organic matter. *J. Mar. Syst.*, A general study of the Spanish North Atlantic boundaries: an interdisciplinary approach 54, 3–37. <https://doi.org/10.1016/j.jmarsys.2004.07.003>.
- Peterson, B.J., Fry, B., 1987. Stable isotopes in ecosystem studies. *Annu. Rev. Ecol. Systemat.* 18, 293–320. <https://doi.org/10.1146/annurev.es.18.110187.001453>.
- Ponchon, A., Grémillet, D., Doligez, B., Chambert, T., Tveraa, T., González-Solís, J., Boulinier, T., 2013. Tracking prospecting movements involved in breeding habitat selection: insights, pitfalls and perspectives. *Methods Ecol. Evol.* 4, 143–150. <https://doi.org/10.1111/j.2041-210x.2012.00259.x>.
- Potapov, A.M., Tiunov, A.V., Scheu, S., 2019. Uncovering trophic positions and food resources of soil animals using bulk natural stable isotope composition. *Biol. Rev.* 94, 37–59. <https://doi.org/10.1111/brv.12434>.
- Raji, O., Dezileau, L., Von Grafenstein, U., Niazi, S., Snoussi, M., Martinez, P., 2015. Extreme sea events during the last millennium in the northeast of Morocco. *Nat. Hazards Earth Syst. Sci.* 15, 203–211. <https://doi.org/10.5194/nhess-15-203-2015>.
- Rathbun, G.B., Scott, N.J., Murphey, T.G., 2002. Terrestrial habitat use by pacific pond turtles in a mediterranean climate. *Southwest. Naturalia* 47, 225–235. <https://doi.org/10.2307/3672910>.
- Ridderinkhof, H., 1998. Sediment transport in intertidal areas. In: *Intertidal Deposits*. CRC Press.
- Robinson, B.W., Wilson, D.S., 1998. Optimal foraging, specialization, and a solution to Liem's paradox. *Am. Nat.* 151, 223–235. <https://doi.org/10.1086/286113>.
- Schwemmer, P., Garthe, S., 2008. Regular habitat switch as an important feeding strategy of an opportunistic seabird species at the interface between land and sea. *Estuar. Coast Shelf Sci.* 77, 12–22. <https://doi.org/10.1016/j.ecss.2007.08.017>.
- Shaiek, M., RoMdhan, M.S., Loc'h, L., 2015. Étude du régime alimentaire de l'ichtyofaune du lac ichkeul (Tunisie), Study of the ichthyofauna diet in the Ichkeul Lake (Tunisia). *Cybiu* 39, 193–210.
- Sheaves, M., 2009. Consequences of ecological connectivity: the coastal ecosystem mosaic. *Mar. Ecol. Prog. Ser.* 391, 107–115. <https://doi.org/10.3354/meps08121>.
- Soulsbury, C.D., Gray, H.E., Smith, L.M., Braithwaite, V., Cotter, S.C., Elwood, R.W., Wilkinson, A., Collins, L.M., 2020. The welfare and ethics of research involving wild animals: a primer. *Methods Ecol. Evol.* 11, 1164–1181. <https://doi.org/10.1111/2041-210X.13435>.
- Spalding, M.D., McIvor, A.L., Beck, M.W., Koch, E.W., Möller, I., Reed, D.J., Rubinoff, P., Spencer, T., Tolhurst, T.J., Wamsley, T.V., van Wesenbeeck, B.K., Wolanski, E., Woodroffe, C.D., 2014. Coastal ecosystems: a critical element of risk reduction. *Conserv. Lett.* 7, 293–301. <https://doi.org/10.1111/conl.12074>.
- Speybroeck, J., Beukema, W., Bok, B., Van Der Voort, J., 2018. Guide Delachaux des amphibiens & reptiles de France et d'Europe. Delachaux et Nieslé 432p.
- Stănescu, F., Iosif, R., Székely, D., Székely, P., Roşioru, D., Cogălniceanu, D., 2013. Salinity tolerance in *Pelobates fuscus* (laurenti, 1768) tadpoles (Amphibia: pelobatidae). *Trav. Muséum Natl. Hist. Nat. Grigore Antipa* 56, 103–108. <https://doi.org/10.2478/travmu-2013-0008>.
- Thirion, J.-M., 2014. Salinity of the reproduction habitats of the western spadefoot toad *Pelobates cultripes* (cuvier, 1829), along the atlantic coast of France. *HERPETOZOA* 27, 13–20.
- Vander Zanden, M.J., Rasmussen, J.B., 1999. Primary consumer  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  and the trophic position of aquatic consumers. *Ecology* 80, 1395–1404. <https://doi.org/10.2307/177083>.
- Verdolin, J.L., 2006. Meta-analysis of foraging and predation risk trade-offs in terrestrial systems. *Behav. Ecol. Sociobiol.* 60, 457–464. <https://doi.org/10.1007/s00265-006-0172-6>.
- Zhang, X., Fichot, C.G., Baracco, C., Guo, R., Neugebauer, S., Bengtsson, Z., Ganju, N., Fagherazzi, S., 2020. Determining the drivers of suspended sediment dynamics in tidal marsh-influenced estuaries using high-resolution ocean color remote sensing. *Remote Sens. Environ.* 240, 111682 <https://doi.org/10.1016/j.rse.2020.111682>.
- Zuur, A.F., Ieno, E.N., Walker, N., Saveliev, A.A., Smith, G.M., 2009. Mixed Effects Models and Extensions in Ecology with R, Statistics for Biology and Health. Springer, New York, NY. <https://doi.org/10.1007/978-0-387-87458-6>.