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# **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>

a. Centre d'Etudes Biologiques de Chizé (CEBC), UMR 7372 du CNRS - La Rochelle Université, 79360 Villiers-en-Bois, France

b. LPO France, Fonderies Royales, 17300 Rochefort, France

c. Réserve naturelle de Moëze-Oléron, LPO, Plaisance, 17780 Saint-Froult, France

d. Réserve naturelle du marais d'Yves LPO, Ferme de la belle espérance, 17340 Yves, France

e. Littoral Environnement et Sociétés (LIENSs), UMR 7266 CNRS-La Rochelle Université, 2 rue Olympe de Gouges, 17000 La Rochelle, France

f. Institut Universitaire de France (IUF), 1 rue Descartes 75005 Paris, France

Correspondence: [lea.lorrain-soligon@cebc.cnrs.fr](mailto:lea.lorrain-soligon@cebc.cnrs.fr)

ID ORCID: 0000-0002-8723-7478

Full postal address : Centre d'Etudes Biologiques de Chizé (CEBC), 405 route de Prissé la Charrière, 79360 Villiers-en-Bois, France

ORCIDs

LLS: 0000-0002-8723-7478

FR : 0000-0003-0232-1142

PB : 0000-0003-3877-9390

YC : 0000-0001-9469-9489

FB : 0000-0002-5788-1326

**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.

**Key-words:** isotopic niche, osmolality, *Pelobates cultripes*,  $\delta^{15}\text{N}$ ,  $\delta^{13}\text{C}$ ,  $\delta^{34}\text{S}$ .

## **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 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, *Pelobates 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 7/10/2020 to 23/10/2020 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 Figure 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 Figure 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 (Speybroeck et al., 2018). Characteristics of body mass and size of the individuals are given in Table S1. 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  $\leq 150 \mu\text{l}$ , 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  $-20^{\circ}\text{C}$  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  $\sim 0.3 \text{ mg}$  (for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) and  $\sim 0.8 \text{ mg}$  (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  $< 0.15 \text{ ‰}$  for both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values, and  $< 0.20 \text{ ‰}$  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 ( $< 200 \text{ m}$ ) 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 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, Fig. S1). 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. 4AB, 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. 4CD, Table 3).

Overlaps between the close, intermediate and distant areas were smaller in MO than in MY (Table S2).

#### 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 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 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 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.

## **Declarations**

### **Declaration of competing interest**

The authors declare that they have no conflict of interest.

### **Author contributions**

LLS, FR & FB have conceptualized the study. LLS, FR, VL, MJ & FB participated in data collection. LLS, FR & FB analyzed the data. LLS, FR, PB, YC & FB interpreted the results. LLS, FR & FB wrote the initial draft. All authors have reviewed and edited the manuscript and approved the final version.

### **Ethics statement**

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

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#### **Data availability statement**

Data will be made available on the corresponding author upon reasonable request.

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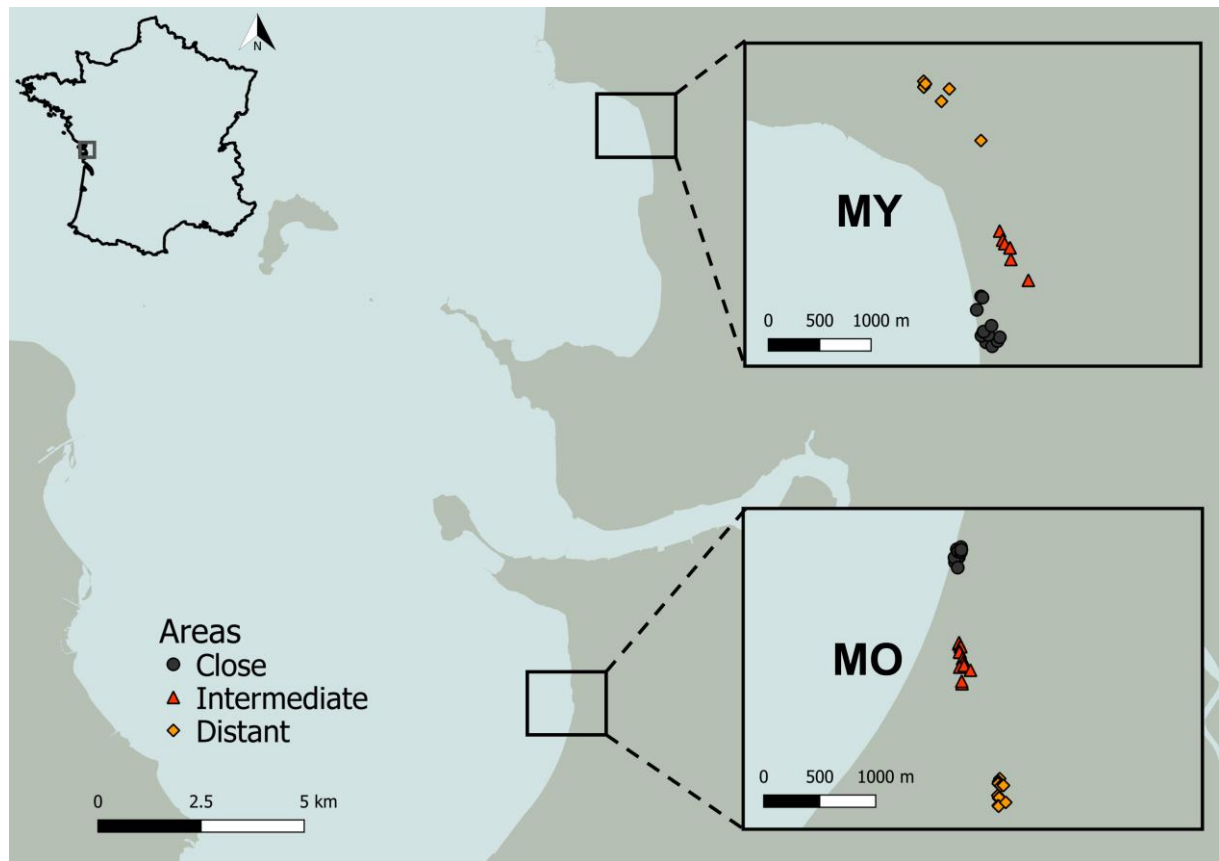


Figure 1: Map of Western France including the two study sites and the position of the captured *Pelobates cultripipes* in the close, intermediate and distant areas from the shore at each site. MO: Réserve Naturelle Nationale de Moëze-Oléron, and MY: Réserve Naturelle Nationale du marais d'Yves.

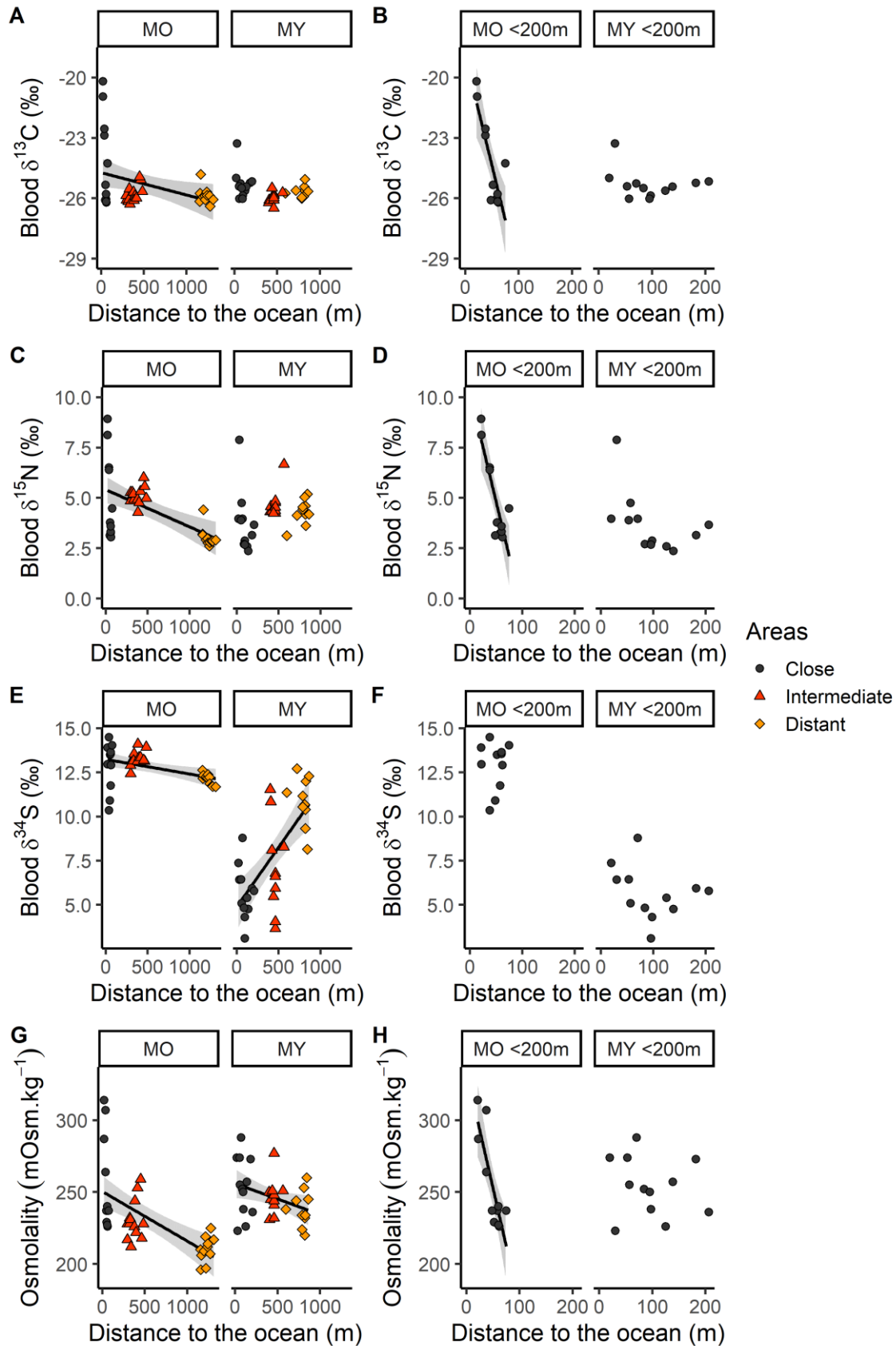


Figure 2: Blood  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ,  $\delta^{34}\text{S}$  values, and plasma osmolality in relation to distance to the ocean across the whole study sites (left column) and restricted to the closest area (right column). Regression lines are used for significant relationships 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).

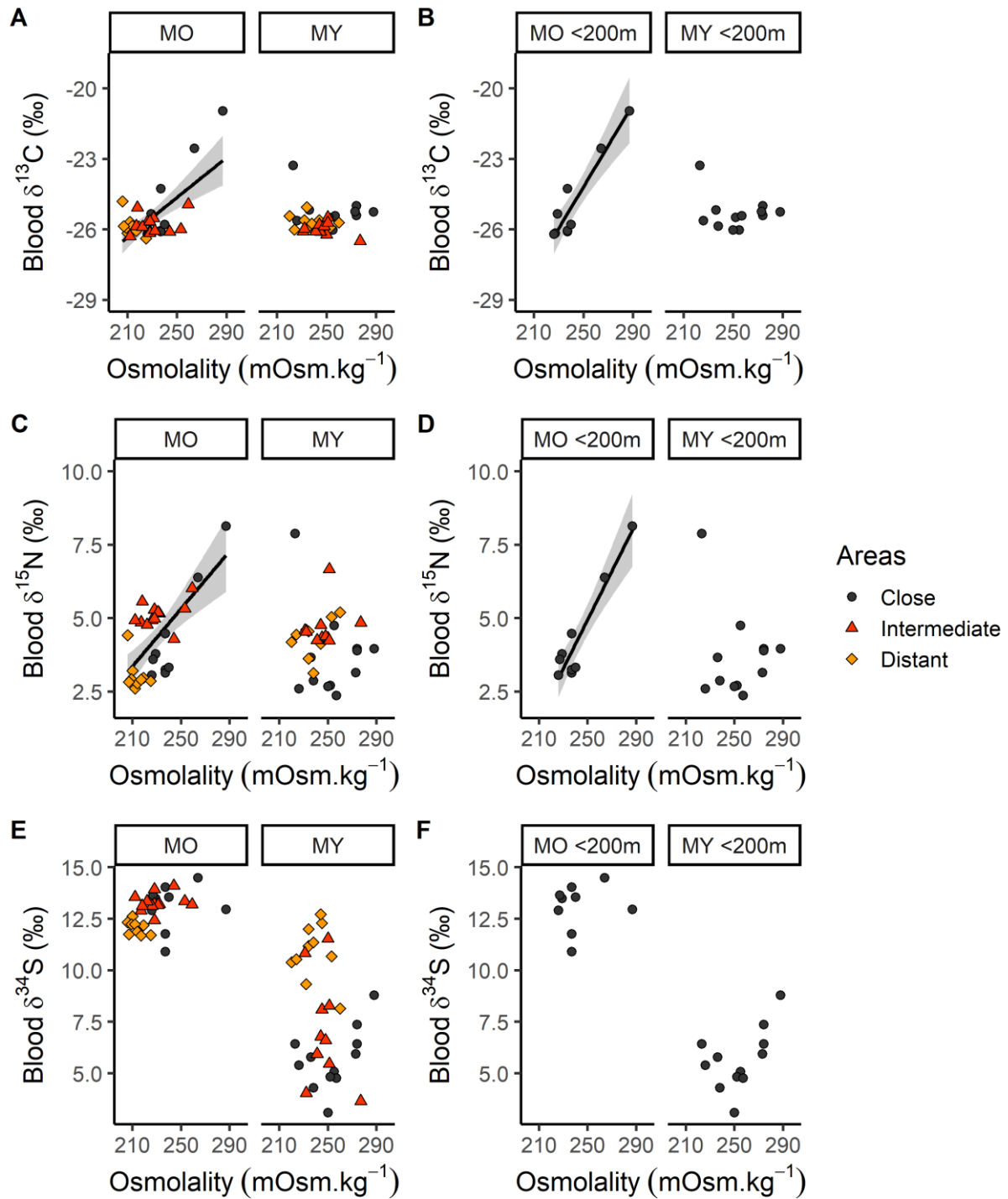


Figure 3: Correlations between blood  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ,  $\delta^{34}\text{S}$  values, and plasma osmolality across the whole study sites (left column) and restricted to the closest area (right column). Regression lines are used for significant relationships solely. MO: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

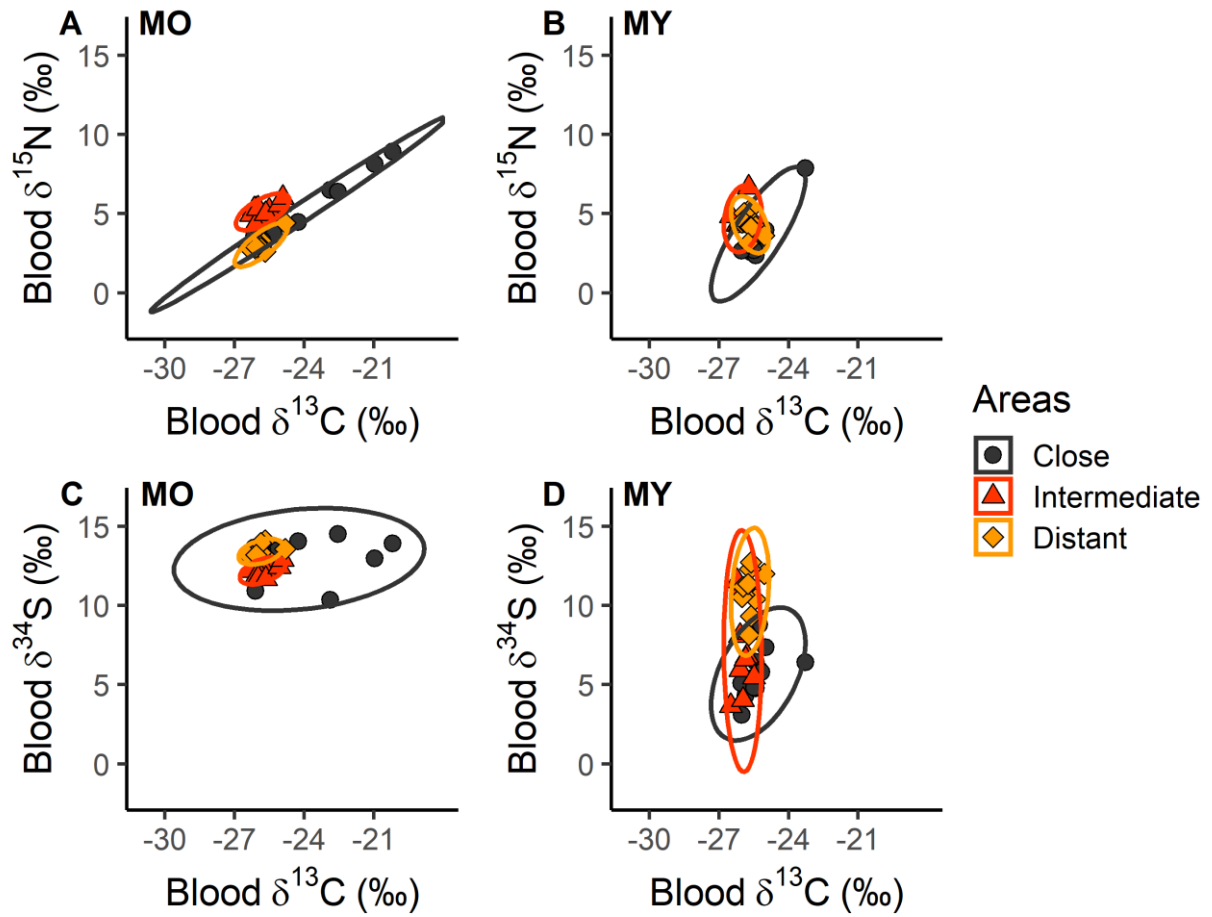


Figure 4: Blood isotopic niches for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  (upper panels) and  $\delta^{13}\text{C}$  and  $\delta^{34}\text{S}$  (lower panels) in MO (left column) and MY (right column) as determined using SIBER. MO: Réserve Naturelle Nationale de Moëze-Oléron and MY: Réserve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

Table 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 <sup>2</sup>	Estimate	SE	t	p	R <sup>2</sup>	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		0.325	0.125	2.607	0.035		-0.003	0.004	-0.838	0.422
		Size	0.901	0.246	0.097	2.551	0.038	-0.028	-	-	-	-
		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		0.264	0.105	2.504	0.041		-0.013	0.007	-1.846	0.095
		Size	0.924	0.205	0.082	2.512	0.04	0.179	-	-	-	-
		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

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

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



## Supplementary material

Table S1. Characteristics of size and body mass for adults in MO and MY for each class of distance. Note that these values cannot be considered as representative of individuals of the population, as we collected blood on large individuals only. MO: Réserve Naturelle Nationale de Moëze-Oléron and MY: Réserve Naturelle Nationale du marais d'Yves.

<i>n</i>		Site	Distance to the ocean	Sex	Size (mm)			Weight (g)		
					Average	Min	Max	Average	Min	Max
<i>n</i> =11	<i>n</i> <sub>females</sub> =8	MO	<200	F	69.19	55	83.5	35.25	17	50.5
	<i>n</i> <sub>males</sub> =3	MO	<200	M	59	56	63	21.33	18	27
<i>n</i> =13	<i>n</i> <sub>females</sub> =9	MO	200-600	F	68.56	57	85	36	22	58
	<i>n</i> <sub>males</sub> =4	MO	200-600	M	60.5	58	66	25.75	24	27
<i>n</i> =11	<i>n</i> <sub>females</sub> =11	MO	>600	F	76.41	70	84	47.95	31	60
	<i>n</i> <sub>males</sub> =0	MO	>600	M	-	-	-	-	-	-
<i>n</i> =12	<i>n</i> <sub>females</sub> =8	MY	<200	F	61.5	54	68	29.625	24.5	37.5
	<i>n</i> <sub>males</sub> =4	MY	<200	M	60.5	59	64	27.5	24	32
<i>n</i> =10	<i>n</i> <sub>females</sub> =7	MY	200-600	F	66.57	59	75	32.5	24.5	46
	<i>n</i> <sub>males</sub> =3	MY	200-600	M	60.67	55	67	23.5	19	28.5
<i>n</i> =10	<i>n</i> <sub>females</sub> =9	MY	>600	F	68.61	62	76	35.06	24	47
	<i>n</i> <sub>males</sub> =1	MY	>600	M	69	-	-	35	-	-

Table S2. Overlaps between ellipses of subpopulations, computed using 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), between 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.

Overlap		MO	MY
Blood $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$	Close-Intermediate	<0.001	0.134
	Close-Distant	0.146	0.174
	Intermediate-Distant	<0.001	0.449
Blood $\delta^{13}\text{C}$ and $\delta^{34}\text{S}$	Close-Intermediate	0.045	0.298
	Close-Distant	0.042	0.036
	Intermediate-Distant	0.075	0.292

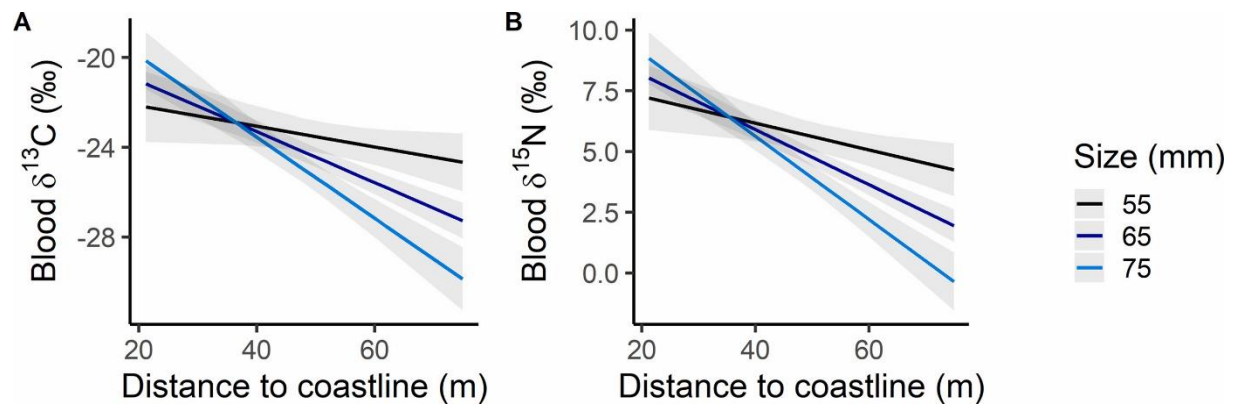


Figure S1. Variations in blood  $\delta^{13}\text{C}$  (A) and  $\delta^{15}\text{N}$  (B) values in relation to distance to the ocean and individual size in the closest area in MO (Réserve Naturelle Nationale de Moëze-Oléron).