Identifying critical habitat of the endangered vaquita (*Phocoena sinus*) with regional $\delta^{13}$C and $\delta^{15}$N isoscapes of the Upper Gulf of California, Mexico

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**ABSTRACT**

The vaquita (*Phocoena sinus*) is the world’s most endangered cetacean and has experienced a 60% reduction in the size of its population in the past decade. Knowledge of its basic ecology is essential for developing successful management plans to protect and conserve this species. In this study, we identified vaquita foraging areas by creating an isoscape of the Upper Gulf of California (UGC) based on sediment and zooplankton carbon ($\delta^{13}$C) and nitrogen ($\delta^{15}$N) isotope values. Our results confirm that this species is confined to the western region of the UGC, which is characterized by relatively high $\delta^{15}$N values (sediments: $10.2^{\%} \pm 2.0^{\%}$, zooplankton: $15.8^{\%} \pm 1.3^{\%}$), higher sea surface temperatures ($\sim 16^{\circ}C$–$25^{\circ}C$), higher concentrations of silt in sediments, and the highest turbidity. In contrast, the eastern region of the UGC had relatively low sediment ($7.7^{\%} \pm 2.4^{\%}$) and zooplankton ($14.6^{\%} \pm 1.0^{\%}$) $\delta^{15}$N values, and the highest concentrations of sand in sediments. Our approach is an effective use of marine isoscapes over a small spatial scale (<200 km) to identify the environmental characteristics that define the critical habitat for an extremely endangered marine mammal.

Key words: marine mammals, stable isotopes, porpoises, marine isoscapes, vaquita, *Phocoena sinus*.

The vaquita (*Phocoena sinus*) is the most endangered marine mammal in the world (Baillie *et al*. 2008, DOF 2010, Gerrodette *et al*. 2011) with an estimated population of 30–60 individuals that is declining by $\sim 18.5\%$ each year (CIRVA 2016). This cetacean has the smallest geographic range of any marine mammal, living only in the waters of the Upper Gulf of California (UGC), Mexico (DOF 2010, Gerrodette *et al*. 2011). Since the first intensive first intensive study of its distribution in the

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2Deceased.
early 1980s (Brownell 1986), nearly all sightings of the vaquita have been in the western half of the UGC (Gerrodette et al. 1995, 2011; Barlow et al. 1997).

The vaquita is a unique species distinguished from other phocoenids by its ability to tolerate a wide range of seasonal fluctuations in temperature, salinity, and other environmental conditions that likely prevailed in the UGC before the Colorado River was extensively dammed in the mid-20th century (Silber and Norris 1991). Even today, the UGC experiences more extreme temperatures (>30°C in the summer warmer months), salinity (36.7–38.8 PSU), and tidal ranges (~7–8 m) than other coastal regions inhabited by phocoenids (Lavín et al. 1997, 1998, 2004; Álvarez and Jones 2002; Sánchez-Velasco et al. 2012). In addition, the UGC shows unusual cyclonic circulation throughout most of the year (Álvarez-Borrego et al. 1975, Carriquiry and Sánchez 1999), which results in (1) net transport of ocean sediments from southeast to northwest along the Sonoran coast to the estuarine basin in the extreme northwest of the UGC and (2) sediment transport from north to south along the Baja California coast (Carriquiry and Sánchez 1999, Carriquiry et al. 2001). The effect of cyclonic circulation is evident in spatial gradients in chlorophyll a concentration, which indicates higher productivity in western vs. eastern UGC (Santamaria-del-Ángel et al. 1994, Millan-Nuñez et al. 1999). Also, microheterotrophic production in the western estuarine basin is higher than that reflected by chlorophyll a concentrations (17.6 mgC m⁻³ h⁻¹), indicating that secondary heterotrophic bacteria production depends on carbon sources other than phytoplankton, such as sedimentary organic matter (Orozco-Borbon 1995).

Despite the vaquita’s imperiled conservation status, we lack information about how such environmental variability in the UGC influences its habitat quality and, by extension, its foraging ecology. Unfortunately, the vaquita’s small population size, small body size, and low surface profile make it difficult to conduct observation-based studies (Silber and Norris 1991, Gerrodette et al. 1995, Barlow et al. 1997, Jaramillo-Legorreta et al. 1999, D’Agrosa et al. 2000), which has impeded our understanding of its spatial movements, distribution, and foraging behavior.

An approach that has been widely employed to track the movements of top marine predators is to compare their isotopic composition to that of marine isoscapes, which most commonly portray the distribution of carbon (δ¹³C) and nitrogen (δ¹⁵N) isotope values at the base of the food web (e.g., phytoplankton or zooplankton) (Graham et al. 2010). Isoscapes for the UGC have not been developed; however, the complexity of this region’s oceanography and proximity to the Colorado River Delta likely result in significant variation in baseline isotopic gradients over relatively small spatial scales (<200 km). Generally, δ¹⁵N values in the UGC are higher than in the southern Gulf of California (Aguíñiga-García 1999), likely due to greater influence in the north of upwelled ¹⁵N-enriched nitrate that has been subject to denitrification at depth (Carriquiry and Sánchez 1999, Carriquiry et al. 2001). This latitudinal pattern may also be influenced by the Colorado River, which likely supplies ¹⁵N-enriched nutrients to the base of the food web (Aguíñiga-García 1999). Thus, the δ¹⁵N of primary producers in the UGC is dependent on the isotopic composition of their nutrient sources (NO₃, NH₄, N₂) and the degree to which they are influenced by microbially mediated processes (e.g., denitrification) (Montoya 2007, Graham et al. 2010), as well as the extent of vertical mixing of the water column that is driven by upwelling/downwelling conditions. Spatial variation in baseline δ¹³C values is associated with a variety of abiotic and biotic factors, including the concentration of dissolved CO₂ (Rau et al. 1992, Goericke and Fry...
1994, Hobson et al. 1994, Graham et al. 2010), phytoplankton cell size, phytoplankton community composition and growth rate (Fry and Wainright 1991, Popp et al. 1998), as well as the influence of $^{13}$C-enriched sources of organic carbon from coastal macrophytes or terrestrial ecosystems (Michener and Schell 1994, France 1995).

Our objective was to characterize the preferred habitat of the vaquita by comparing its isotope composition to a $\delta^{13}$C and $\delta^{15}$N isoscape of the UGC that we created from an extensive set of sedimentary and biological samples collected across multiple trophic levels. This approach enabled us to identify the environmental characteristics that define the vaquita’s critical habitat, and we anticipate that our $\delta^{13}$C and $\delta^{15}$N isoscape can be used to evaluate habitat preferences for other endemic and endangered species in this region, such as the totoaba (*Totoaba macdonaldi*).

**METHODS**

**Field Data and Sample Collection**

During two oceanographic cruises on the R/V *Francisco de Ulloa* in June 2010 and March 2011, we recorded physical data and retrieved sediment, phytoplankton, and zooplankton samples for carbon ($\delta^{13}$C) and nitrogen ($\delta^{15}$N) isotope analysis; sample sizes and scientific names are shown in Table 1. The sample area extends northwest from San Felipe, Baja California (31°01′39″N, 114°50′07″W) to Puerto Peñasco, Sonora (31°19′00″N, 113°32′13″W). We collected sediment from only 52 stations in June 2010, but phytoplankton and zooplankton ($n = 152$ stations) samples were collected from hydrographic stations in both June 2010 and March 2011.

Depth profiles of temperature (T) and salinity (S) were measured using a SeaBird SBE-911Plus CTD (conductivity, temperature, depth) with sensors for dissolved oxygen ($O_{2D}$), turbidity (TBDZ), and chlorophyll a (Chl “a”) concentrations, Salinity (PSU, UNESCO 1981) data were processed using the manufacturer’s software (Godínez et al. 2011). We collected the upper ~1 cm of sediment using a Van Veen drag. Phytoplankton and zooplankton were collected during 5 min surface hauls using conical nets with 64 and 500 μm mesh sizes, respectively. In addition, we collected muscle samples from gastropods (*Atrina* sp.) and bivalves (*Nassarius* sp.) near a small island in the western UGC (Rocas Consag) and at a fish market in the nearby town of San Felipe, Baja California. Fish from the genera *Mugil*, *Anchoa*, *Oligoplectes*, and *Cynoscion* were collected by line fishing and through donation or purchase from local fishermen (Table 1). These samples were collected for later comparison with $\delta^{13}$C and $\delta^{15}$N values of invertebrates and fish collected from Adair Bay in the eastern UGC reported in Spackeen (2009). All biological samples were stored at −20°C until processing for stable isotope analysis. Vaquita bone collagen samples were obtained from the dorsal section of the skull of 15 stranded individuals archived in the marine mammal collection at the Research Center for Food and Development (CIAD) in Guaymas, Sonora; these individuals were opportunistically collected from 1983 to 1993.

**Sample Processing**

Sediment samples were demineralized with repeated rinses of 0.2 N hydrochloric acid to remove calcium carbonate, then rinsed 2–3 times with distilled water and dried at 60°C (Bouillon et al. 2002). Approximately 100 g of each of the 52
Table 1. Mean (± SD) δ\(^{13}\)C and δ\(^{15}\)N values of sediment and biological samples collected in the Western and Eastern regions of the Upper Gulf of California.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Western</th>
<th>Eastern</th>
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<tr>
<td></td>
<td>N</td>
<td>δ(^{13})C ± SD (‰)</td>
</tr>
<tr>
<td>Sediment</td>
<td>21</td>
<td>-21.4 ± 3.4</td>
</tr>
<tr>
<td>Phytoplankton</td>
<td>42</td>
<td>-20.1 ± 1.5</td>
</tr>
<tr>
<td>Zooplankton</td>
<td>42</td>
<td>-19.2 ± 0.8</td>
</tr>
<tr>
<td>Chione</td>
<td></td>
<td>—</td>
</tr>
<tr>
<td>Atrina</td>
<td>4</td>
<td>-18.3 ± 1.5</td>
</tr>
<tr>
<td>Nassarius</td>
<td>3</td>
<td>-14.9 ± 0.7</td>
</tr>
<tr>
<td>Anchoa</td>
<td>2</td>
<td>-16.2 ± 1.6</td>
</tr>
<tr>
<td>Cynoscion</td>
<td>3</td>
<td>-15.1 ± 0.8</td>
</tr>
<tr>
<td>Mugil</td>
<td>2</td>
<td>-12.5 ± 2.4</td>
</tr>
<tr>
<td>Oligoplis</td>
<td>4</td>
<td>-16.2 ± 1.5</td>
</tr>
<tr>
<td>P. sinus</td>
<td>15</td>
<td>-12.1 ± 0.7</td>
</tr>
</tbody>
</table>

aData from Spackeen (2009).
sediment samples were sent to the Edaphology Laboratory at the Centro de Investigaciones Biológicas del Noroeste (CIBNOR) in La Paz, Baja California Sur, for granulometric analysis following the methods described in Taylor (1948). The phytoplankton and zooplankton samples were placed separately in Petri dishes and demineralized using repeated rinses of 0.2 N hydrochloric acid, then rinsed with distilled water and dried at 60°C (Chanton and Lewis 1999). All bivalves and fish were identified to the lowest possible taxonomic level. We then used a scalpel to subsample a ~10 mg section of bivalve adductor muscle, gastropod foot, or dorsal fin muscle from fish, and all samples were dried at 60°C for stable isotope analysis. Vaquita bone fragments were demineralized in a solution of 0.5 N hydrochloric acid for 12–24 h and then dried at 60°C. We did not lipid-extract biological samples due to its potential effects on δ15N values observed in some marine and freshwater organisms (Post et al. 2007, Logan et al. 2008, Hoffman and Sutton 2010). However, all muscle and bone collagen samples we analyzed had a weight percent carbon to nitrogen concentration ratio (C/N) of <3.5, which is within the range of pure protein (Ambrose 1990).

All sedimentary and biological samples were ground to a fine powder, and ~25 mg of sediment and ~1 mg of muscle or bone collagen were weighed into tin capsules for δ13C and δ15N analysis at the University of California Davis Stable Isotope Laboratory using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer. Stable isotope data are expressed as δ values using the equation δ13C or δ15N = 1,000 × (Rsample − Rstandard/Rstandard), where RSample and RStandard are the ratios of the heavy to light isotope (e.g., 13C/12C or 15N/14N) for each sample and standard, respectively. Delta (δ) units are reported as parts per thousand or per mil (‰). Laboratory standards were calibrated against NIST Standard Reference Materials IAEA-N1, IAEA-N2, IAEA-N3, USGS-40, and USGS-41, and had a within-run standard deviation of ±0.2‰ for δ13C and ±0.3‰, for δ15N. These standards were calibrated to the internationally accepted standards of Vienna PeeDee Belemnite (V-PDB) for δ13C and atmospheric nitrogen (AIR) for δ15N.

**Statistical Analysis of Environmental and Stable Isotope Data**

We used a Student’s t-test (STATISTICA 8.0), to assess whether phytoplankton and zooplankton collected in June 2010 and March 2011 had different δ13C and δ15N values.

**Carbon and nitrogen isoscapes**—We generated isoscapes with SURFER software v.9.0 (Radabaugh et al. 2013, Fourqurean et al. 2015) based on sediment (Fig. 1A, B), phytoplankton (Fig. S2), and zooplankton (Fig. 2) δ13C and δ15N values to visualize spatial gradients in the baseline isotopic composition of the UGC. We used ordinary point kriging with a linear variogram model, and exact interpolation as a gridding method for the data.

Statistical analysis was performed using a complete linkage clustering and a dissimilarity matrix based on Euclidean distance to find the greatest differences between groups. The matrix contained isotope data that included data for zooplankton (n = 104, June 2010 and March 2011) and sediment (n = 52, June 2010) to obtain equal sample (site) sizes for these two substrates; data were normalized using z scores. Phytoplankton isotope data were highly variable and were not included in the cluster analysis; the high degree of isotopic variation in phytoplankton likely reflects ephemeral environmental conditions and its relatively short incorporation
rate in comparison to sediment and zooplankton samples that integrate over longer time periods. Previous authors have noted similar patterns between phytoplankton and zooplankton isotope values and thus chose to use the latter for constructing marine isoscapes (Graham et al. 2010, McMahon et al. 2013).

Cluster analysis was performed using the furthest neighbor sorting method with PRIMER software (v6.1.4). The optimal grouping in the dendrogram (Fig. S1) was determined with the silhouettes widths method, which is a measure of the degree of

Figure 1. Surface sediment $\delta^{13}$C (A) and $\delta^{15}$N (B) isoscapes for the Upper Gulf of California (UGC). Small black circles represent sampling stations and the black polygon represent the vaquita refuge in the western UGC. (C) Location of regions identified by cluster analysis; blue circles represents the Eastern region and green circles represents the Western region. Location of sampling stations are indicated by solid dots (black, green and blue). The number of vaquita sightings in the last three decades (Jaramillo-Legorreta 2008) are noted by the size of the triangles.
membership of an object to its cluster. Values of the silhouette coefficient range from −1 to +1: values close to +1 indicate that a sample is distinct from neighboring clusters; a value of 0 indicates that the sample is on or very close to the decision boundary between two neighboring clusters; and negative values indicate that those samples might have been assigned to the wrong cluster (Rousseeuw 1987, Borcard et al. 2011). To detect significant differences among the dissimilarity of the regions obtained from the cluster, we applied a one-way ANOSIM as a nonparametric, multiresponse permutation test using PRIMER software v6.1.4. More positive R-values in an ANOSIM test indicate stronger separation. Finally, results of the cluster analysis were included on a map generated with SURFER (v9.0) of the locations of historical vaquita sightings in the UGC (Fig. 1C).

**Environmental Features of the UGC**

A principal component analysis (PCA; ter Braak 1986) was used to assess the relationship between environmental variables and the two distinct isotopic zones (eastern and western) identified in the cluster analysis (Fig. S1). The PCA was calculated with an ordering method on a covariance matrix, using environmental variables collected from 52 sites in June 2010 and March 2011; data included in the PCA were not normalized. We used a Type 1 scaling method where distances between objects in the biplot (Fig. S3) represent an approximation of their Euclidean distances in
multidimensional space. Environmental data consisted of mean values for station depth (m), temperature (°C), salinity (PSU), chlorophyll a (mgC m⁻³ h⁻¹), turbidity (NTU), dissolved oxygen (mL/L), and the grain size of sediment samples.

**Vaquita’s Foraging Area**

We then compared vaquita isotope values with data from the two distinct isotopic zones (eastern and western) based on sediment and zooplankton isotope data. We also compared vaquita data with our macrofauna isotope data collected from Rocas Consag in the western UGC (31°07'00''N, 114°29'00''W) and with data from Bahia Adair in the eastern UGC reported by Spackeen (2009) (Table 1, Fig. 3).

**RESULTS**

**June and March δ¹³C and δ¹⁵N Variation in the UGC**

The hierarchical cluster analysis defined two distinct geographical zones (Fig. S1) based on sediment and zooplankton isotope values (Fig. 1–3; ANOSIM test, R = 0.351). The two regions included the “western” zone adjacent to the Baja California Peninsula, and the “eastern” zone adjacent to the Sonoran coastline (Fig. 1C). Phytoplankton isotope values were more variable over time and had no clear geographical pattern (Fig. S2).

*Figure 3.* Mean δ¹³C and δ¹⁵N values of sediment and biological samples collected from the Upper Gulf of California; error bars represent standard deviation. Open and closed symbols are samples collected from the Eastern and Western regions, respectively, which are connected by dashed lines. Labels are associated with samples from the Eastern region, including sediment (Sed), phytoplankton (Phy), zooplankton (Zoo), *Chione* (Chi), *Atrina* (Atr), *Nassarius* (Nss), *Magil* (Mgl), *Anchoa* (Ach), *Oligoplicites* (Olg), *Cynoscion* (Cyc). The white circle represents mean trophic-corrected δ¹³C and δ¹⁵N values for vaquita; we subtracted 3‰ and 2‰ from measured δ¹³C and δ¹⁵N, respectively, to account for trophic discrimination (Borrell et al. 2012).
The current distribution of the vaquita lies within the western zone (Fig. 1C), which was characterized by relatively high mean (± SD) sediment (10.2 ± 2.0%; Fig. 1B, C) and June (15.9 ± 1.2%) and March (15.8 ± 1.3%) zooplankton δ15N values. In contrast, the eastern zone had lower sediment and zooplankton δ15N values during both sampling periods (Table 2). We did not observe any significant differences in sediment (t = 1.4, df = 52, P = 0.18) or zooplankton δ13C values collected in June (t = −1.6, df = 52, P = 0.12) between the western and eastern zones, but we did observe significant differences in zooplankton δ13C values collected in March between these two zones (t = 2.2, df = 52, P = 0.03). For δ15N, we found significant differences in sediment (t = 4.1, df = 52, P < 0.01) and zooplankton samples collected in June (t = 3.9, df = 50, P < 0.01) and March (t = 4.9, df = 50, P < 0.01) between the western and eastern zones.

### Environmental and Isotopic Patterns in the UGC

We used a PCA that incorporated hydrological and sedimentological data from both sample periods to define each geographic zone (Table S1). About 84% of the variance in the variables was explained by the first two axes. This analysis clearly identified the western zone based on shallow depth, as well as sediment silt and clay content (Table S1), while the eastern zone was primarily correlated with deeper depths and sandier sediments (Table S1).

Sea surface temperature ranged from 20.4°C to 24.1°C in June 2010 and from 15.8°C to 19.0°C in March 2011. Higher temperatures were recorded in the western zone, which has the shallowest average water depth in the study area (Table S2). The lowest temperature was recorded in the eastern zone, which has the deepest average water depth in the study area. Mean salinity (June: 36.2 PSU and March: 35.8 PSU) was high across the UGC, but the western zone had higher salinities than the eastern zone during both sampling periods. Mean dissolved oxygen values were also high throughout the study area; June: 4.4 ± 0.3 mL/L and March: 5.4 ± 0.5 mL/L. Mean chlorophyll a concentrations ranged from 0.03 to 0.7 mg C m−3 h−1 in June and from 0.0 to 7.5 mg C m−3 h−1 in March. Turbidity was highest in the western zone during both months (June 2010 and March 2011), which may be associated with the predominance of silt-clay sediments in this region, in contrast to higher proportions of fine sand in the eastern zone (Table S2).

Although isotopic and hydrological differences were consistent between zones for June and March, we identified some significant temporal variation when analyzing the combined isotopic data set of phytoplankton and zooplankton collected from both the eastern and western UGC. First, phytoplankton collected during March (−19.5‰ ± 1.2‰) had significantly higher mean (±SD) δ13C values than

<table>
<thead>
<tr>
<th>Region</th>
<th>N</th>
<th>δ13C ± SD (%oo)</th>
<th>δ15N ± SD (%oo)</th>
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<tbody>
<tr>
<td></td>
<td>Sediment June</td>
<td>Sediment June</td>
<td>Zooplankton March</td>
</tr>
<tr>
<td>Western</td>
<td>33</td>
<td>−20.7 ± 4.7</td>
<td>−18.8 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>−23.7 ± 2.5</td>
<td>−19.3 ± 0.9</td>
</tr>
<tr>
<td>Eastern</td>
<td></td>
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phytoplankton collected in June \((-21.4\%_{\text{oo}} \pm 1.1\%_{\text{oo}}\) (Fig. S2; \(t = 9.92, \text{df} = 148, P < 0.01\)). Zooplankton collected during March \((-19.2\%_{\text{oo}} \pm 1.1\%_{\text{oo}}\) also had significantly higher mean (±SD) \(^{13}\)C values than zooplankton collected during June \((-20.1\%_{\text{oo}} \pm 1.2\%_{\text{oo}}\) (Fig. 2A, B; \(t = 5.20, \text{df} = 149, P < 0.01\)). A similar pattern was observed in \(^{15}\)N, as phytoplankton collected in March \((13.3\%_{\text{oo}} \pm 1.2\%_{\text{oo}}\) had higher values than samples collected in June \((12.0\%_{\text{oo}} \pm 1.6\%_{\text{oo}}\) (Fig. S2; \(t = 5.40, \text{df} = 148, P < 0.01\)). We did not find significant differences between \(^{15}\)N values of zooplankton collected in June 2010 and March 2011 (Fig. 2C, D; \(t = 0.75, \text{df} = 149, P = 0.46\)).

\(^{13}\)C and \(^{15}\)N Values of Invertebrates and Fish

We did not statistically assess differences in invertebrates or fish collected in the two zones due to low sample sizes for most genera. Table 1 and Figure 3 compare \(^{13}\)C values in the western vs. eastern zones, including sediments (+3.5\%_{\text{oo}}), phytoplankton (+0.5\%_{\text{oo}}), zooplankton (+1.1\%_{\text{oo}}), benthic bivalves (Chione: +3.1\%_{\text{oo}} and Atrina: +6.4\%_{\text{oo}}), gastropods (Nassarius: +4.1) and most species of fish: Cynoscion: +4.1%_{oo}, Mugil: +4.3%_{oo}, Oligoplicites: +4.8%_{oo} and Paralabrax: +4.1%_{oo}. Anchovies are one exception and samples collected in the western and eastern UGC had similar \(^{13}\)C and \(^{15}\)N values. For \(^{13}\)C, the pattern was less consistent but in most cases samples collected in the eastern zone had slightly higher \(^{13}\)C values than their counterparts in the western zone, including Anchovia (+1.3\%_{oo}) Cynoscion (+0.8\%_{oo}), and Oligoplicites (+2.0\%_{oo}). In contrast, phytoplankton (+1.1\%_{oo}) and zooplankton (+0.5\%_{oo}) had slightly higher \(^{13}\)C values in the western vs. eastern zone. Mean \(^{13}\)C values for mullet (Mugil), the bivalves (Chione and Atrina), and the gastropods (Nassarius) were similar between zones (Table 1).

Unfortunately, we could only locate 15 vaquita specimens for stable isotope analysis. However, given the low estimated population size (\(n = 60\); CIRVA 2016), we believe that our sample size is representative of the species. Although the vaquita bone collagen samples were derived from a mixture of juveniles (\(n = 4\)), adults (\(n = 7\)), and individuals of undetermined age (\(n = 4\)), we did not find any significant differences in \(^{13}\)C or \(^{15}\)N values (Kruskal–Wallis test, \(H = 1.18, \text{df} = 2, P = 0.56; H = 0.07, \text{df} = 2, P = 0.97\) among females (\(n = 5\)), males (\(n = 7\)), and individuals of unknown sex (\(n = 3\)) \((H = 0.06, \text{df} = 2, P = 0.97; H = 3.67, \text{df} = 2, P = 0.16\)). Overall, vaquita had mean (±SD) \(^{13}\)C and \(^{15}\)N values of \(-12.4\%_{\text{oo}} \pm 0.5\%_{\text{oo}}\) and \(+20.8\%_{\text{oo}} \pm 0.8\%_{\text{oo}}\), respectively (Table 1). To account for trophic discrimination and directly compare vaquita isotope data to that of its potential prey (Fig. 3), we subtracted 3\%_{oo} and 2\%_{oo} from measured vaquita mean \(^{13}\)C and \(^{15}\)N values, respectively (Borrell et al. 2012).

**DISCUSSION**

Our study identified two distinct geographic zones (western and eastern) in the Upper Gulf of California (UGC) based on sediment, zooplankton, and macrofauna \(^{13}\)C and \(^{15}\)N values. The largest number of vaquita sightings occurs in the western zone (Barlow 1986; Brownell 1986; Silber 1990; Gerrodette et al. 1995, 2011; Barlow et al. 1997; Gallo-Reynoso 1998; Jaramillo-Legorreta et al. 1999; Vidal...
et al. 1999; Jaramillo-Legorreta 2008), and comparison of isotope data from vaquita to that from sediments, zooplankton, invertebrates, and fish (potential prey) also support the hypothesis that the preferred habitat of this species is in the western portion of the UGC.

Hydrological and biogeochemical characteristics also differ between the western and eastern zones of the UGC. Specifically, the western zone is characterized by higher sediment and zooplankton $\delta^{15}N$ values, and has a shallower mean depth, higher turbidity, and a higher proportion of silt (Fig. 3). In contrast, the eastern zone had lower sediment and zooplankton $\delta^{15}N$ values, a greater mean depth, and a higher proportion of very fine sand and lower turbidity (Table S1). Spatial variation in these hydrological and physical characteristics likely influences the recycling of organic matter at the base of the food web, and likely helps maintain the observed differences in isotope values between geographic zones. Carriquiry et al. (2001) argued that due to the structure of the Colorado River Delta, sediments in the northwest portion of the UGC are derived from sediment resuspension and organic matter remineralization, and that these recycled sediments are dispersed across the UGC basin via cyclonic transport. Given its proximity to the delta, the western zone is influenced more by these hydrological processes, which likely results in higher $\delta^{13}N$ values from the constant recycling and remineralization of organic matter by bacterioplankton (Orozco-Borbon 1995). Higher $\delta^{13}N$ values may also be the result of tidal mixing, which enhances the upwelling of $^{15}N$-enriched nitrate from intermediate depths (~100–200 m) into the euphotic zone (Montoya 2007). Our data for zooplankton and macrofauna at higher trophic levels shows that this enriched baseline $\delta^{15}N$ signature in the western UGC permeates up food webs to top consumers like vaquita.

Other features that defined the western zone were physical characteristics like high turbidity associated with a predominance of silty sediments. Several studies suggest that porpoises are attracted to coastal areas influenced by river discharge that supplies nutrients to the base of the food chain and consequently produces large prey aggregations (Watts and Gaskin 1985, Palka 1995, Read 1999, Ridgway and Harrison 1999). Silber (1991) also argued that high turbidity minimizes detection of small porpoises by potential predators such as larger delphinids and sharks.

Despite the temporal mismatch of when the sediment, biological, and vaquita samples were collected from the UGC in our study, the same pattern of an increase in $\delta^{15}N$ values in the western vs. eastern zones has been observed in previous studies that compare sediment isotope values to those of California sea lions (Zalophus californianus) in the UGC (Aguíniga-García 1999, Porras-Peters et al. 2008, Aurioles-Gamboa et al. 2013). In addition, riverine inputs as measured by the (overall low) discharge of the Colorado River, as well as temperature and salinity in the UGC (Godínez 1997, Lavín et al. 1998), did not differ when the vaquita (1983–1993), sedimentological (2010–2011), and invertebrate (2007–2011) samples were collected. Thus, we are confident that the observed UGC isoscape, especially for $\delta^{15}N$, is temporally consistent and could be a valuable tool for identifying critical habitats of top consumers from both modern and historical contexts in the UGC. However, potential interannual variation in baseline isotope values should be considered in future studies.

We did not find any significant differences in sediment or zooplankton $\delta^{13}C$ values collected from the western and eastern zones, which is mirrored in some, but not all, higher trophic level organisms collected from these localities. $\delta^{13}C$
values for the gastropod, *Nassarius*, and the mullet, *Mugil*, collected from the two geographic zones were similar, but other bivalves and fish species had higher $\delta^{13}C$ values in the eastern zone by $\sim 0.7\%_o - 2.0\%_o$ in comparison to the western zone. This pattern is presumably driven by high local biomass of $^{13}C$-enriched macroalgae in Adair Bay, Sonora where invertebrates and fish were collected from the eastern zone (Spackeen 2009). Bivalves (*Atrina*), gastropods, and most fish collected in the western zone had much higher $\delta^{15}N$ values by $\sim 4.1\%_o - 6.4\%_o$ (Fig. 3, Table 1) in comparison to their counterparts in the eastern zone; a similar pattern observed, to a lesser degree, in sediment and zooplankton. The only exception was *Anchoa*, which had similar $\delta^{15}N$ values in both geographic zones, which likely indicates that this species regularly moves between the eastern and western portions of the UGC.

This study demonstrates the utility of using high-resolution isoscapes over small spatial scales ($< 200$ km) for understanding the distribution, movement, and foraging behavior of an elusive and highly endangered top predator that has a small geographic range. In addition, our approach could be applied to study resource and habitat use of other top marine consumer that live at the interface of marine and freshwater aquatic habitats characterized by large gradients in baseline isotope values over small spatial scales. Finally, our study supports previous findings that suggest that the conservation of the critically endangered vaquita should focus on reducing its interactions with fisheries, but also preserving the integrity of its habitat in the western portion of the UGC.

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**Supporting Information**

The following supporting information is available for this article online at http://onlinelibrary.wiley.com/doi/10.1111/mms.12483/suppinfo.

*Figure S1.* Cluster analysis of groups of sampling stations defined by sediment and zooplankton $\delta^{13}$C and $\delta^{15}$N data collected in the Upper Gulf of California during June (2010) and March (2011).

*Figure S2.* Isoscapes of $\delta^{13}$C (A and B) and $\delta^{15}$N (C and D) values of phytoplankton samples collected in June (2010) and March (2011). Small black circles represent sampling stations and the black polygon represents the vaquita refuge.

*Figure S3.* Principal component analysis of hydrogeological and sedimentological data collected in the Upper Gulf of California. Black triangles represent the Western isotopic zone and white triangles the Eastern isotopic zone. The vectors represent the variables used in the analysis: turbidity (TBDZ), silt-clay (LA), silt (L), fine sand (MF), very fine sand (AMF), salinity (S), oxygen (O2), temperature (T), depth (P) sampler in June and March.

*Table S1.* Results between sediment and zooplankton $\delta^{13}$C and $\delta^{15}$N values from June (2010) and March (2011) and environmental indicators from the principal component analysis in the Upper Gulf of California. The values in bold are those that best define vaquita habitat according to the PCA.

*Table S2.* Mean environmental variables (with minimum and maximum in parentheses) and grain size of the sediment samples collected in the Upper Gulf of California.