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Dear Editorial Board,

Thank you and both reviewers for the comments on our manuscript “Stable isotopes in barnacles as a tool to understand green sea turtle (*Chelonia mydas*) regional movement patterns”. Our responses are below in **bold**. A marked up manuscript with the changes made since the last submission is attached following these responses.

The coauthors agree with this revision and with this resubmission to *Biogeosciences*.

Kind regards,

Matthias Detjen and coauthors

Anonymous Referee #2

The study described in this manuscript investigates variation in isotopes of oxygen and carbon in the shells of barnacles that live in association with sea turtles. The objective is to match isotopic variation in the shells with predicted values in the Pacific Ocean to assess the range of migration by the host turtles. Though not the first time this idea has been proposed, the study provides potentially new insight on the movement of sea turtles in the Pacific. However, in my opinion there are a few significant core issues and a number of technical editing corrections that need to be addressed before this manuscript is ready for publication.

1. A clearer description of where shell material was taken from the barnacles is needed. Using barnacle anatomical terms would help (e.g. base [bottom], aperture [top], paries [wall plate]). If I understand correctly the milled sections were taken at distances measured from the base of a paries. Was material used from the surface or deeper within the paries? Was sampling done in the middle of the paries or at the lateral edge? [this makes a difference since the paries have a growing margin along the base and along their sides]? Were the barnacles of similar size (i.e. age?) The size range of 1.5 – 2.5 mm is mentioned in the discussion but reporting sizes of specimens in the results section would be useful. It should also be noted that nothing is known about growth rates in this species of barnacle.

This is correct, the material was taken along the outer facing surface of the paries in distances measured from the base of the paries along the axis of growth. We selected barnacles that were of similar sizes with the following barnacle sizes separated by respective turtles: (i) GD42 were 1.6 mm, 1.3 mm and 1.6 mm, (ii) GI 41 were 1.6 mm, 2.2 mm, and 2.5 mm, and (iii) GI 43 were 2.0 mm, 2.1 mm and 1.6 mm. We added a sentence clarifying our lack of knowledge about the growth rates of this species.

2. I did not understand the number of samples (rows) reported in table 1. From the text the authors state that 9 barnacle samples from 3 different turtles were ultimately analyzed so I would expect either 3 or 9 rows of data but the table reports 6 rows of data. The mismatch needs

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clarification. It would also be most useful to arrange the rows of data by each turtle sampled and either list the number of barnacles sampled for each turtle or list each sample in its own row (9 rows isn't much more than 6).

We reconfigured the table to show three rows each with the averages of three barnacles per turtle. It now shows the distance from paries' base, oxygen isotope ratio and carbon isotope ratio in the barnacles collected from three green sea turtles (GD42, GI41 & GI43). Distance is given in millimeters and isotope ratios are reported versus the VPDB scale

Distance from Base			$\delta^{18}\text{O}$ Concentration			$\delta^{13}\text{C}$ Concentration		
<i>GD42</i>	<i>GI41</i>	<i>GI43</i>	<i>GD42</i>	<i>GI41</i>	<i>GI43</i>	<i>GD42</i>	<i>GI41</i>	<i>GI43</i>
0.350	0.350	0.350	-1.359	-1.343	-1.310	0.729	-0.451	-0.299
0.719	0.727	0.743	-1.283	-1.220	-1.431	0.798	-0.398	-0.619
1.052	1.135	1.107	-1.414	-1.168	-1.200	0.624	-0.124	-0.914
1.403	1.559	1.451	-1.500	-1.097	-1.160	1.090	0.009	-0.481
1.550	1.937	1.725	-1.503	-1.004	-1.476	1.430	-0.002	0.096
n/a	2.354	2.067	n/a	-1.321	-1.379	n/a	0.227	-0.811

3. The authors make the link with isotopic ratios and water temperature but doesn't salinity also affect isotopic ratios? Maybe salinity is uniform enough that it is of no concern but possibilities for its influence should be discussed. Also more explanation is needed on the parameters and formula used for the paleotemperature equation (after Epstein et al. 1953?) and is this based on parameters for mollusk shells or modified for barnacles (sensu Killingley & Newman 1982 [should be cited]) as discussed in Killingley & Lutcavage 1983?

Epstein, S., R. M. Buchsbaum, H. A. Lowenstam, and H. C. Urey. 1953. Revised carbonate-water isotopic temperature scale. *Bulletin of the Geological Society of America* 64:1315-1326.

Killingley, J. S., and W. A. Newman. 1982. ^{18}O fractionation in barnacle calcite: a barnacle paleotemperature equation. *Journal of Marine Research* 40:893-902.

Our study uses the adjusted paleotemperature equation as shown in Killingley & Newman (1982) and we will cite the paper directly to make this clear. Salinity affects the isotopic ratios of the water and we capture the varying salinity in the Pacific through the seawater $\delta^{18}\text{O}$ parameter in the paleotemperature equation, using the dataset published in LeGrande and Schmidt (2006).

Killingley, J. S., and W. A. Newman. 1982. ^{18}O fractionation in barnacle calcite: a barnacle paleotemperature equation. *Journal of Marine Research* 40:893-902.

LeGrande, A. N. and Schmidt, G. A.: Global gridded data set of the oxygen isotopic composition in seawater, *Geophysical Research Letters*, 33, L12604, 2006.

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Would it be possible for figures 1 and 2 to show multiple solid line isopleths (contours) of temperature (or oxygen isotope ratios) along with the shaded predicted migration region?

Figures edited as requested.

4. Technical edits:

Pg. 4656 Line 23 . . . migration patterns, as well as fine-scale . . .

Edited

Pg. 4657 Line 12 Because of their intimate connections, species that are associates of particular hosts have been used . . .

Edited

Line 22 As obligate commensals, these barnacles . . .

Edited

Pg. 4658 Line 6-9 This sentence does not read well. Perhaps splitting it into two would help.

Changed sentence to: These movements can be traced by comparing barnacle oxygen isotope ratios to mapped prediction for these values. Temporal reconstruction could potentially also be added as our understanding of the pace at which successive barnacle calcite layers are laid down improves.

Line 13 “would have” this phrase does not make sense to me

Edited

Line 14. . .in the barnacle *Platylepas hexastylus*, an epibiont of turtles, collected . . .

Edited

Pg. 4659 Line 5 It is not customary to cite conference proceedings. I suggest using “(unpublished data)” in place of Gomez et al.

Changed to “personal observation” as this statement better corresponds to an observation.

Line 9 “axis of growth” rather than “growth trajectory”

Edited

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Line 11 I don't know what is meant by "endoskeleton". Inner layer of shell? Barnacles have a thin exoskeleton around their body but no endoskeleton.

This was referring to the paries and corrected accordingly.

Pg. 4660 Line 10 "the edge" does this mean basal margin?

Yes, it does and this was corrected in the text.

Lines 11-13 growth axis of the barnacle shell not the barnacle

Edited

Line 14 Do you have a reference to cite for the Vienna Pee Dee Belemnite scale?

Added citation.

Line 19 . . . spanned three orders of magnitude . . .

Edited

Interactive comment on Biogeosciences Discuss., 12, 4655, 2015.

1

2 **Title:** Stable isotopes in barnacles as a tool to understand green sea turtle (*Chelonia mydas*)
3 regional movement patterns

4

5 **Running page head:** Isotopes in sea turtle barnacles

6

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21 ABSTRACT: Sea turtles are migratory animals that travel long distances between their feeding and
22 breeding grounds. Traditional methods for researching sea turtle migratory behavior have
23 important disadvantages, and the development of alternatives would enhance our ability to
24 monitor and manage these globally endangered species. Here we report on the isotope signatures
25 in green sea turtle (*Chelonia mydas*) barnacles (*Platylepas* sp.) and discuss their potential relevance
26 as tools with which to study green sea turtle migration and habitat use patterns. We analyzed
27 oxygen ($\delta^{18}\text{O}$) and carbon ($\delta^{13}\text{C}$) isotope ratios in barnacle calcite layers from specimens collected
28 from green turtles captured at the Palmyra Atoll National Wildlife Refuge (PANWR) in the Central
29 Pacific. Carbon isotopes were not informative in this study. However, the oxygen isotope results
30 suggest likely regional movement patterns when mapped onto a predictive oxygen isotope map of
31 the Pacific. Barnacle proxies could therefore complement other methods in understanding regional
32 movement patterns, informing more effective conservation policy that takes into account
33 connectivity between populations.

34

35

36 KEY WORDS: *Chelonia mydas*, [Platylepas](#) barnacle, epibiont, proxy, oxygen isotope, carbon isotope

37 1. INTRODUCTION

38 Long distance migratory behavior between breeding and feeding grounds, a key component of sea
39 turtle ecology, creates important research and conservation challenges (Godley et al., 2010).
40 Understanding migratory and habitat use patterns is a critical step in the design of comprehensive
41 conservation and management strategies aimed at protecting all of a species' range, including the
42 corridors connecting distant habitats. For many sea turtle populations we lack detailed
43 spatiotemporal knowledge about ~~migrations~~migration patterns, as well as a fine-scale
44 understanding of habitat use. This dearth of information may hinder conservation efforts, especially
45 in scarcely studied areas such as the Central Pacific (Wallace et al., 2010).

46 Previous studies on sea turtle movement patterns have been based on mark-recapture, satellite
47 telemetry, or genetic analysis (Godley et al., 2010). Although these methods have provided key
48 insights, they also have important shortcomings. Mark-recapture can have very low return rates
49 (Oosthuizen et al., 2010). Satellite telemetry is a very effective method for tracking turtles across
50 long distances but can be prohibitively expensive, and loss and malfunction of transmitters is
51 common (Hays et al., 2007; Hebblewhite and Haydon, 2010). Genetic studies can be a very effective
52 way of delineating population structure and natal origin, but are uninformative about movements
53 after the sea turtles hatch (Bowen and Karl, 2007). ~~Additional~~Therefore, ~~additional~~ methods are
54 needed to help us map patterns of movement and habitat use at scales useful for conservation
55 planning (Godley et al., 2010).

56 ~~Because of the close associations between associate~~ Because of their intimate connections, species
57 ~~and their that are associates of particular~~ hosts, they have been used as proxies for the study of host
58 ecology, demography, and evolutionary history (Nieberding and Olivieri, 2007). Recent research
59 has shown that studying associate species such as parasites and commensals, can be a cost-effective
60 alternative to ecological research on the host themselves (Byers et al., 2011; Hechinger et al., 2007).

61 Several barnacle species are commonly found in sea turtles, attached to the skin and shell.

62 Barnacles are found in the majority of green turtles ~~we~~ observed in [a long-term study of marine](#)
63 [turtles at](#) Palmyra Atoll National Wildlife Refuge (Gómez, A., personal observation), and they have
64 been reported widely from sea turtle populations from across the world (Casale et al., 2004; Frick
65 et al., 2010; Rawson et al., 2003; Schwartz, 1960; Torres-Pratts et al., 2009; Zardus and Balazs,
66 2007). ~~Generally considered symbionts. As obligate commensals,~~ these barnacles form close, long-
67 lasting associations with their hosts, and may thus provide useful information about turtle ecology.

68 Previous studies have shown that isotopes in barnacle calcite can be used to reconstruct migratory
69 patterns and habitat use in California gray whales (Killingley, 1980) and loggerhead turtles
70 (Killingley and Lutcavage, 1983). Isotope ratios in calcite layers can be used to approximate the
71 water temperature throughout the life of individual barnacles because warmer waters have
72 reduced oxygen ratios (Killingley and Lutcavage, 1983), where the oxygen isotopes in the barnacle
73 calcite fractionate or change in relative proportion during calcite formation depending on the
74 oxygen ratios in the surrounding water (Kendall and Caldwell, 1998). Therefore, oxygen isotope
75 ratios obtained from barnacles can be informative about turtle movements at large scales, as long
76 as those movements occurred along water temperature gradients (Killingley and Lutcavage, 1983).
77 These movements can be traced by comparing barnacle oxygen isotope ratios to mapped prediction
78 for these values ~~with the addition of a potential temporal.~~ [Temporal](#) reconstruction [could](#)
79 [potentially also be added](#) as ~~we get a better our~~ understanding of the pace at which successive
80 barnacle calcite layers are laid down [improves](#). Carbon isotope ratios can be expected to vary as
81 microhabitats differ in the concentration of dissolved carbon, and can therefore provide
82 information about habitat occupancy across sites, with lagoons and the pelagic zone assumed to
83 have low and high carbon conditions respectively (Killingley and Lutcavage, 1983). ~~These carbon~~
84 ~~results would have allowed us to understand which habitat the turtles predominantly inhabit. Here~~
85 ~~we report on oxygen ($\delta^{18}\text{O}$) and carbon ($\delta^{13}\text{C}$) isotopes in barnacles~~ [Here we report on oxygen](#)

86 [\(\$\delta^{18}\text{O}\$ \) and carbon \(\$\delta^{13}\text{C}\$ \) isotopes in the barnacle *Platylepas hexastylus*, an epibiont of turtles.](#)

87 collected from green sea turtles (*Chelonia mydas*) at Palmyra Atoll National Wildlife Refuge in the
88 Central Pacific and discuss the potential of this method as a tool with which to study sea turtle
89 movements.

91 2. MATERIALS AND METHODS

92 The barnacle specimens used in the experiment were collected at Palmyra Atoll National Wildlife
93 Refuge (PANWR; 05° 52' N, 162° 05' W), central Pacific Ocean. The atoll has a wide shallow reef,
94 extensive reef terraces at both the eastern and western ends, and three lagoons (Collen et al., 2009).
95 The islets and 12 nautical miles of the surrounding ocean have been designated a marine protected
96 area by the U.S. Fish and Wildlife Service since 2001. In 2005, the Center for Biodiversity and
97 Conservation of the American Museum of Natural History initiated a research and conservation
98 program for sea turtles at PANWR. The program includes research into the turtles' distribution and
99 abundance, connectivity, feeding ecology, health, and threats (McFadden et al., 2014; Sterling et al.,
100 2013). The sea turtle population at this site has been studied using mark-recapture, satellite
101 telemetry, and genetic analysis (Sterling et al., 2013).

102 *Platylepas* sp. barnacles were collected from adult green sea turtles caught in PANWR during the
103 summer of 2011. These barnacles were ~~exclusively found embedded in the turtles' soft tissue~~
104 ~~(Gómez et al., 2011)~~ found embedded in the turtles' soft tissue (A. Gómez, personal observation).

105 Barnacles were removed from the turtle's skin and stored in vials with 90% ethanol until analysis.
106 We analyzed a total of 12 barnacles. In order to assess the consistency of recorded isotope ratios of
107 different barnacles from a given turtle we sampled three barnacles ~~from each per turtle~~. The
108 barnacles were dissected and milled along their ~~axis of growth trajectory~~ using a Merchantek
109 MicroMill (Electro Scientific Industries, Inc., Portland, United States) ~~to take calcite samples~~. The

110 mill was programmed to ~~take~~make passes [on the outer facing surface of the paries](#) perpendicular to
111 the [axis of growth trajectory of the endoskeleton that were in distances](#) 0.3-0.4 mm apart. ~~The~~
112 ~~samples~~[For each sample, a record was kept of the distance along the growth axis from barnacles'](#)
113 [base to where each pass had been made. Samples](#) were taken from the outermost part of the
114 ~~endoskeleton~~[paries](#) to exclude any calcite deposits that might have been the result of ageing and
115 thickening of the individual plates. [It should be noted that nothing is known about growth rates in](#)
116 [this species of barnacle.](#) The calcite samples were sent to the Keck Paleoenvironmental &
117 Environmental Stable Isotope Laboratory at the University of Kansas, where they were analyzed for
118 oxygen ($\delta^{18}\text{O}$) and carbon ($\delta^{13}\text{C}$) stable isotope ratios. A Kiel Carbonate Device III and a Finnigan
119 MAT253 isotope ratio mass spectrometer (Finnigan MAT, Bremen, Germany) were used to perform
120 the laboratory analyses.

121 Oxygen isotope ratios in barnacle calcite can be expected to vary predictively as a function of the
122 water's oxygen isotope ratios and temperature and can be solved for using a conversion formula
123 [\(Killingley and Lutcavage, 1983\)](#) ~~(Epstein et al., 1953)~~ [with a required modification for barnacle](#)
124 [calcite \(Killingley and Newman, 1982\).](#) We reversed the formula by rearranging variables for the
125 water's oxygen isotope ratio, [which accounts for variations in salinity,](#) and temperature to create a
126 map of predicted barnacle oxygen isotope ratios. We used annual average sea surface temperature
127 data from NOAA's World Ocean Database (NOAA, 2005) for the temperature variable in the
128 equation, and published water oxygen isotope figures from 2006 (LeGrande and Schmidt, 2006) as
129 inputs in the equation. The resulting map allowed us to put the oxygen isotope results from the
130 barnacles into geographic context. We used this map to create an isoscape, thereby defining the
131 largest possible area from which the isotope values measured from the calcite could have
132 accumulated across the life of the barnacles sampled. A detailed methodology is included as an
133 electronic supplement.

134

135 **3. RESULTS**

136 Because some of the calcite samples were not sufficiently large to be analyzed with precision in the
137 mass spectrometer, we obtained a complete set of results for barnacles from two of the four sea
138 turtles sampled and only partial results from one other. We included nine barnacles from three
139 turtles in our analysis, as results from the fourth were too incomplete. [The selected barnacles on](#)
140 [the respective turtles had the following sizes measured from the base to the aperture: \(i\) 1.6 mm,](#)
141 [1.3 mm and 1.6 mm on GD42, \(ii\) 1.6 mm, 2.2 mm, and 2.5 mm on GI41, and \(iii\) 2.0 mm, 2.1 mm](#)
142 [and 1.6 mm on GI43.](#) A summary of the stable isotope ratios are reported in Table 1. The youngest
143 part of the barnacle is that closest to the [edge with the last growth](#)[basal margin](#) or [terminal edge of](#)
144 [bottom, as](#) the barnacle, ~~as it~~ grows outward. These isotope ratios represent the values across the
145 growth ~~trajectory~~[axis](#) of the barnacle [shell](#) going from the youngest to the oldest part of the
146 barnacle. The carbon and oxygen isotope ratios are reported versus the Vienna Pee Dee Belemnite
147 (VPDB) scale ([Coplen, 1995](#)), which is used as benchmark value. The maps predicting calcite
148 oxygen isotope ratios in the Central Pacific showed uniform ratios along the equator and steep
149 gradients towards northern and southern latitudes.

150

151 Oxygen isotope ratios in our calcite samples did not show major fluctuations throughout the life of
152 the barnacle, while the carbon isotope ratios of the barnacles spanned [a wide range](#)[three orders](#) of
153 [values](#)[magnitude](#). The highest measured oxygen isotope ratio in the collected barnacles was -0.951
154 $\delta^{18}\text{O}$. We used this value as a contour to create an envelope in which we would expect our sea
155 turtles to have stayed throughout the lifetime of the barnacle (Fig. 1). The resulting isoscape
156 included PANWR. We also created a more conservative isoscape that corrected for the fact that the
157 original isoscape maps might be overestimating the isotope ratios. The first step was to identify the
158 expected oxygen isotope ratio at PANWR on the map, as the isotopes in the barnacles' youngest

159 layer would be expected to coincide with it. The map predicted a calcite oxygen isotope ratio of -
160 $1.08075 \delta^{18}\text{O}$, while the average youngest layers of the barnacles collected were $-1.34337 \delta^{18}\text{O}$,
161 giving a difference of $0.262 \delta^{18}\text{O}$. Adding this difference to the original isoscape value of $-0.951 \delta^{18}\text{O}$
162 gave a corrected calcite oxygen ratio of $-0.689688 \delta^{18}\text{O}$. This ratio was then used to produce a larger
163 standardized isoscape delineating the sea turtles movements during the barnacles' lifetime (Fig. 2).

164

165

166 4. DISCUSSION

167 ~~Oxygen isotope values observed in the barnacles were transformed using the methods in Killingley~~
168 ~~and Lutcavage (1983) with the water oxygen isotope ratios reported for PANWR, which~~
169 ~~Our study found that oxygen isotopes in barnacles' calcite could be used to broadly delineate the area in which~~
170 ~~the sampled sea turtles moved during the life of the barnacles, allowing us to exclude visitation of~~
171 ~~major breeding grounds in the Pacific. Carbon isotopes were not informative in this study and~~
172 ~~assessing their utility as proxies with which to explore sea turtle habitat use requires further study.~~

173 Oxygen isotope values observed in the barnacles in this study indicated that the calcite ratios
174 conform to sea temperatures of 28°C and 30°C . Assuming that average temperatures above 28°C
175 are found in the warmest waters of the Central Pacific that are in proximity to the equator, then our
176 data suggest that turtles did not venture beyond these waters during the lifespan of the barnacles
177 collected. This is consistent with observations from the field, which suggest that turtles spend
178 extended periods of time in PANWR (Sterling et al., 2013).

179 To obtain a more concrete ~~idea~~picture of the sea turtles' movements, we used the predicted calcite
180 oxygen isotope map estimating the area within which the sea ~~turtle~~turtles may have moved. The
181 contour delineating the isoscape of possible movements was large (Fig. 1 and 2) as water

182 temperatures in the Central Pacific are relatively uniform. However, some major known green
183 turtle grounds that are within in the potential migratory range of green turtles from PANWR were
184 not within this isoscape (STC, 2012). These include Ogasawara Island (Japan), NW Australia and
185 Hawaii, which also remain outside of the boundary when using the more conservative adjusted
186 oxygen isoscape. Importantly, recent research shows that the natal origin of sea turtles in PANWR
187 can almost exclusively be found to the West and South of the Central Pacific (Naro-Maciel et al.,
188 2014). Therefore, the boundaries we delineate in this study: 1) include PANWR, 2) are consistent
189 with ecological observation, and 3) are consistent with new genetic evidence about the population
190 structure of green sea turtles at PANWR.

191 Because we cannot exclude the possibility that our isoscapes simply reflect residency at Palmyra,
192 we are unable to quantify the method's utility as an indicator of large scale movements. However,
193 our data suggest that it can be used to delineate envelopes of likely residency across the Pacific
194 basin. Therefore we suggest that this method has the potential to provide valuable data to inform
195 comprehensive management strategies, by helping identify specific ecological and political areas
196 within or outside a given population's range.

197 A wide range in the barnacles carbon isotopes may indicate that turtles made use of a variety of
198 microhabitats around the atoll, possibly moving between areas like the lagoon and the pelagic zone,
199 which are assumed to have low and high carbon conditions respectively (Killingley and Lutcavage,
200 1983). An alternative explanation is that the turtles are frequenting ecologically heterogeneous
201 areas beyond PANWR. However, any conclusions drawn from these results need to be viewed
202 conservatively, as a heterogeneous environment does not necessarily explain the lack of
203 consistency in our data ~~that has~~, which have marked dissimilarities in carbon isotope ratios
204 between barnacles on the same turtle. There could be differences in uptake or expression of carbon
205 isotopes in each barnacle possibly limiting the use of the carbon isotope data in this study system.

206 Previous studies used a larger barnacle species than the ones found on the green turtles at PANWR
207 (Killingley and Lutcavage, 1983). *Platylepas* sp. specimens that we collected had sizes ranging
208 between 1.53 and 2.5 mm, which is a magnitude smaller than the *Chelonibia testudinaria* recovered
209 from loggerhead turtles in previous studies (Killingley, 1980; Killingley and Lutcavage, 1983). This
210 resulted in fewer data points and limited statistical analysis of the results.

211 In summary, this limited dataset suggests that inferences about green sea turtle spatial ecology
212 obtained from isotope analysis are broadly consistent with field observations and genetic analyses.
213 Isotope analysis may provide low-resolution information about sea turtle connectivity, potentially
214 defining areas of interest for research and management. Therefore, we suggest that this method can
215 only complement but not replace other tools to investigate turtle migration and habitat use
216 patterns. One advantage of the method is its low cost. The total cost of analyzing three barnacles on
217 one sea turtle was below 170 USD (56 USD per barnacle in 2011). This makes using barnacle
218 proxies an option that could be explored further in the study of spatial ecology and could be
219 improved in future applications.

220 Future research can add critical information with which to improve this method. We lack basic
221 information about the natural history of many turtle epibionts. Because [we ignore of the dearth of](#)
222 [data on](#) baseline growth rates for *Platylepas* sp., the time span between successive calcite layers is
223 unknown, and therefore the system cannot be attached to an absolute temporal scale. We also lack
224 benchmarks for isotope ratios in barnacles. Therefore, it is difficult to draw conclusions about the
225 significance of fluctuations that we observed, especially for the variation in carbon isotope ratios.
226 The utility of barnacles as proxies of sea turtle movement at study sites such as PANWR might not
227 be fully realized until these key knowledge gaps are addressed.

228

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237 findings, conclusions, and recommendations are those of the author(s) and do not necessarily
238 reflect the views of the National Oceanic and Atmospheric Administration or the U.S. Department of
239 Commerce. We acknowledge the Palmyra Atoll National Wildlife Refuge, U.S. Fish and Wildlife
240 Service, Department of the Interior. This is Palmyra Atoll Research Consortium publication number
241 PARC-~~xxxx~~[0119](#).
242

243 **References**

- 244 Bowen, B. W. and Karl, S. A.: Population genetics and phylogeography of sea turtles, *Molecular Ecology*,
245 16, 4886-4907, 2007.
- 246 Byers, J. E., Altman, I., Grosse, A. M., Huspeni, T. C., and Maerz, J. C.: Using [Parasitic-Trematode](#)
247 [Larvae](#) to [Quantify](#) an [Elusive-Vertebrate-Host](#), *Conservation Biology*, 25, 85-93, 2011.
- 249 Casale, P., Freggi, D., Basso, R., and Argano, R.: Epibiotic barnacles and crabs as indicators of *Caretta*
250 *caretta* distribution and movements in the Mediterranean Sea, *Journal of the Marine Biological*
251 *Association of the United Kingdom*, 84, 1005-1006, 2004.
- 252 Collen, J. D., Garton, D. W., and Gardner, J. P. A.: Shoreline [Changes](#) and [Sediment](#)
253 [Redistribution](#) at Palmyra Atoll (Equatorial Pacific Ocean): 1874–Present, *Journal*
254 *of Coastal Research*, doi: 10.2112/08-1007.1, 2009. 711-722, 2009.
- 255 [Coplen, T.: Reporting of stable hydrogen, carbon, and oxygen isotopic abundances, *Geothermics*, 24,](#)
256 [707-712, 1995.](#)
- 257 [Epstein, S., Buchsbaum, R., Lowenstam, H. A., and Urey, H. C.: Revised carbonate-water isotopic](#)
258 [temperature scale, *Geological Society of America Bulletin*, 64, 1315-1326, 1953.](#)
- 259 Frick, M. G., Zardus, J. D., and Lazo-Wasem, E. A.: A new *Stomatolepas* barnacle species (Cirripedia:
260 Balanomorpha: Coronuloidea) from leatherback sea turtles, *Bulletin of the Peabody Museum of Natural*
261 *History*, 51, 123-136, 2010.
- 262 Godley, B. J., Barbosa, C., Bruford, M., Broderick, A. C., Catry, P., Coyne, M. S., Formia, A., Hays, G. C.,
263 and Witt, M. J.: Unravelling migratory connectivity in marine turtles using multiple methods, *Journal of*
264 *Applied Ecology*, *J. Appl. Ecol.*, 47, 769-778, 2010.
- 265 [Gómez, A., Sterling, E., Lazo-Wasem, E., Arengo, F., K. McFadden, K., and Vintinner, E.: Epibiont](#)
266 [community composition in green turtles in Palmyra Atoll National Wildlife Refuge, *International Sea*](#)
267 [Turtle Society Annual Meeting, San Diego, CA, 2011.](#)
- 268 Hays, G. C., Bradshaw, C. J. A., James, M. C., Lovell, P., and Sims, D. W.: Why do Argos satellite tags
269 deployed on marine animals stop transmitting?, *Journal of Experimental Marine Biology and Ecology*,
270 349, 52-60, 2007.
- 271 Hebblewhite, M. and Haydon, D. T.: Distinguishing technology from biology: a critical review of the use
272 of GPS telemetry data in ecology, *Philosophical Transactions of the Royal Society B: Biological Sciences*,
273 365, 2303-2312, 2010.
- 274 Hechinger, R. F., Lafferty, K. D., Huspeni, T. C., Brooks, A. J., and Kuris, A. M.: Can parasites be indicators
275 of free-living diversity? Relationships between species richness and the abundance of larval trematodes
276 and of local benthos and fishes, *Oecologia*, 151, 82-92, 2007.
- 277 Kendall, C. and Caldwell, E. A.: Fundamentals of isotope geochemistry, *Isotope tracers in catchment*
278 *hydrology*, 1998. 51-86, 1998.
- 279 [Killingley, J. and Newman, W.: 18O fractionation in barnacle calcite: a barnacle paleotemperature](#)
280 [equation, *Journal of Marine Research*, 40, 893-902, 1982.](#)
- 281 [Killingley, J. S.: Migrations of *California* gray whales tracked by \[oxygen\]\(#\)-18](#)
282 [Variations](#) in their epizoid barnacles, *Science*, 207, 759-760, 1980.
- 283 Killingley, J. S. and Lutcavage, M.: Loggerhead turtle movements reconstructed from 18O and 13C
284 profiles from commensal barnacle shells, *Estuarine, Coastal and Shelf Science*, 16, 345-349, 1983.
- 285 LeGrande, A. N. and Schmidt, G. A.: Global gridded data set of the oxygen isotopic composition in
286 seawater, *Geophysical Research Letters*, 33, L12604, 2006.
- 287 McFadden, K. W., Gómez, A., Sterling, E. J., and Naro-Maciel, E.: Potential impacts of historical
288 disturbance on green turtle health in the unique & protected marine ecosystem of Palmyra Atoll
289 (Central Pacific), *Marine pollution bulletin*, 89, 160-167, 2014.

- 290 Naro-Maciel, E., Gaughran, S. J., Putman, N. F., Amato, G., Arengo, F., Dutton, P. H., McFadden, K. W.,
291 Vintinner, E. C., and Sterling, E. J.: Predicting connectivity of green turtles at Palmyra Atoll, central
292 Pacific: a focus on mtDNA and dispersal modelling, *Journal of The Royal Society Interface*, 11, 20130888,
293 2014.
- 294 Nieberding, C. M. and Olivieri, I.: Parasites: proxies for host genealogy and ecology?, *Trends in Ecology &*
295 *Evolution*, 22, 156-165, 2007.
- 296 NOAA: <http://www.nodc.noaa.gov/OC5/indprod.html> last access: 03/04 2012.
- 297 Oosthuizen, W. C., De Bruyn, P., Bester, M. N., and Girondot, M.: Cohort and tag-site-specific tag-loss
298 rates in mark-recapture studies: A southern elephant seal cautionary case, *Marine Mammal Science*, 26,
299 350-369, 2010.
- 300 Rawson, P. D., Macnamee, R., Frick, M. G., and Williams, K. L.: Phylogeography of the coronulid barnacle,
301 *Chelonibia testudinaria*, from loggerhead sea turtles, *Caretta caretta*, *Molecular Ecology*, 12, 2697-2706,
302 2003.
- 303 Schwartz, F.: The barnacle *Platylepas hexastylus* encrusting a green turtle, *Chelonia mydas mydas*, from
304 Chincoteague Bay, Maryland, *Chesapeake Science*, 1, 116-117, 1960.
- 305 STC: <http://www.conserveturtles.org/seaturtlenestingmap.php>, last access: 05/15 2012.
- 306 Sterling, E. and Naro-Maciel, E.: ~~Distribution and abundance of endangered marine turtles at Palmyra~~
307 ~~Atoll, Central Pacific, In Society for Conservation Biology 20th Annual Meeting, San Jose, California,~~
308 ~~2006.~~
- 309 Sterling, E.-J., McFadden, K., Holmes, K., Vintinner, E., Arengo, F., and Naro-Maciel, E.: Ecology and
310 conservation of marine turtles in a foraging ground in the Central Pacific, *Chelonian Conservation*
311 *Biology*, 12, 2-16, 2013.
- 312 Torres-Pratts, H., Scharer, M. T., and Schizas, N. V.: Genetic diversity of *Chelonibia caretta*, commensal
313 barnacles of the endangered hawksbill sea turtle *Eretmochelys imbricata* from the Caribbean (Puerto
314 Rico), *Journal of the Marine Biological Association of the United Kingdom*, 89, 719-725, 2009.
- 315 Wallace, B. P., DiMatteo, A. D., Hurley, B. J., Finkbeiner, E. M., Bolten, A. B., Chaloupka, M. Y.,
316 Hutchinson, B. J., Abreu-Grobois, F. A., Amorocho, D., Bjorndal, K. A., Bourjea, J., Bowen, B. W., Duenas,
317 R. B., Casale, P., Choudhury, B. C., Costa, A., Dutton, P. H., Fallabrino, A., Girard, A., Girondot, M.,
318 Godfrey, M. H., Hamann, M., Lopez-Mendilaharsu, M., Marcovaldi, M. A., Mortimer, J. A., Musick, J. A.,
319 Nel, R., Pilcher, N. J., Seminoff, J. A., Troeng, S., Witherington, B., and Mast, R. B.: Regional ~~management~~
320 ~~units~~Management Units for ~~marine turtles~~Marine Turtles: A novel framework for prioritizing
321 conservation and research across multiple scales, *PLoS ONE*, 5, 2010.
- 322 Zardus, J. D. and Balazs, G. H.: Two previously unreported barnacles commensal with the green sea
323 turtle, *Chelonia mydas* (Linnaeus, 1758), in Hawaii and a comparison of their attachment modes,
324 *Crustaceana*, 80, 1303-1315, 2007.

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Table 1. Distance from [terminal edge paries' base](#), oxygen isotope ratio and carbon isotope ratio in *Platylepas* sp. barnacles collected from [three](#) green sea turtles (GD42, GI41, and GI43) in Palmyra Atoll National Wildlife Refuge. [Rows show the average of three barnacles per turtle sampled.](#) Distance is given in millimeters and isotope ratios are reported versus the VPDB scale.

Average distance (Range)Distance from Base			Average $\delta^{18}\text{O}$ concentration (Range)Concentration			Average $\delta^{13}\text{C}$ concentration (Range)Concentration		
GD42	GI41	GI43	GD42	GI41	GI43	GD42	GI41	GI43
0.350	0.350	0.350	-1.337	-0.907	-	0	-0.451	-0.299
			1.574	1.016	1	2		
			1.136	1.06	3	3		

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Isotopes in sea turtle barnacles

0.729	0.727	0.743	-1.297(-	-0.005(-	-	0.	-0.398	-0.619
(0.65,			1.509,	1.295,	1.	7		
0.835)71			1.018)283	1.212)220	43	9		
9					1	8		
1.098	1.135	1.107	-1.268(-	-0.041(-	-	0.	-0.124	-0.914
(0.95,			1.481,	1.01,	1.	6		
1.215)05			0.985)414	1.125)168	20	2		
2					0	4		
1.471	1.559	1.451	-1.252(-	-1.097	-	1.	0.206(-	-0.481
(1.25,			1.622,		1.	0	0.854,	
1.646)40			1.015)500		16	9	1.328)0.00	
3					0	0		
1.775	1.937	1.725	-1.247(-	-1.004	-	1.	-0.381(-	0.096
(1.55,			1.503,		1.	4	0.36,	
2.005)55			0.951)		47	3	1.43)002	
0					6	0		
n/a	2.21	-1.340(-	n/a	-1.321	-	n/	0.227	-0.419(-
	(2.031,	1.425,-			1.	a		0.811,
	2.459)35	1.217)2.067			37			0.322)
	4				9			

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385 **Fig. 1.** Oxygen isoscape (shaded in gray) showing the area in which we would expect our sea turtles

386 to have resided throughout the life of the barnacles tested. This isoscape was calculated using an

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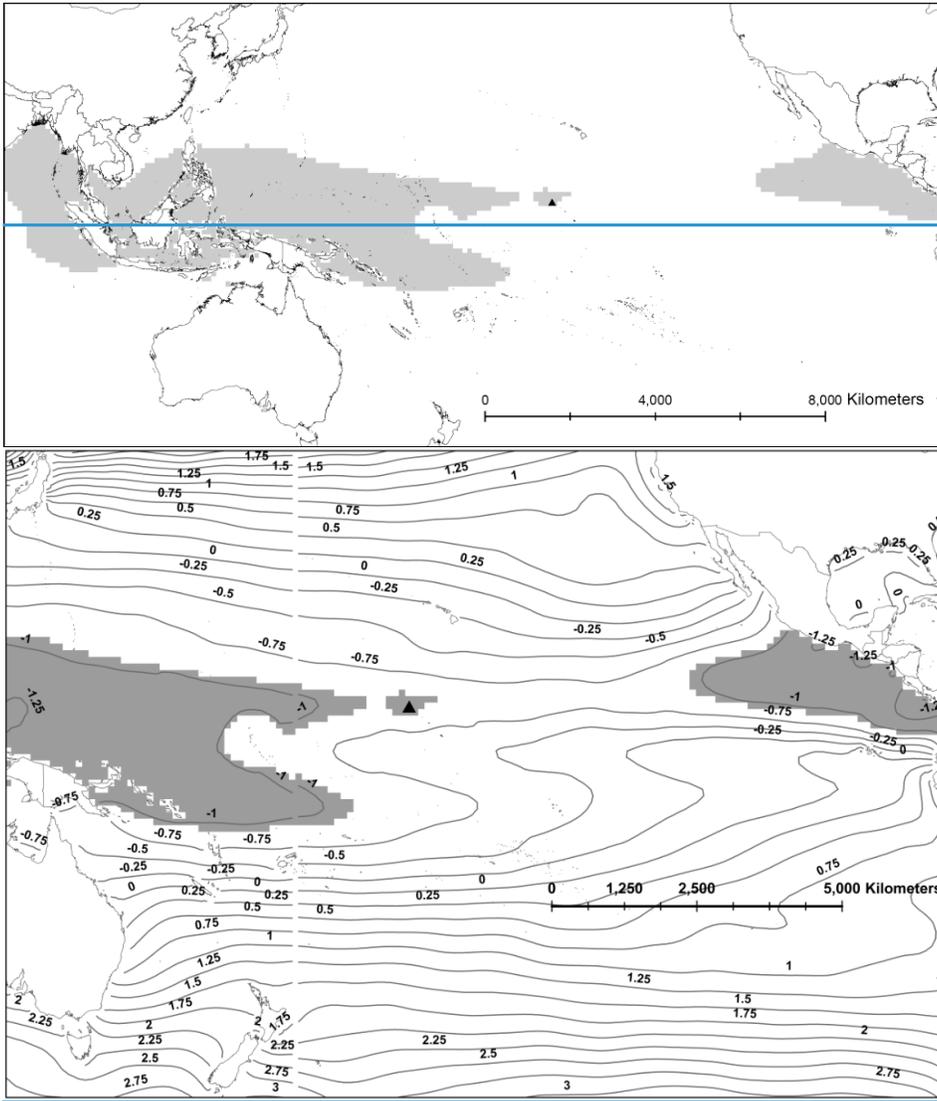
387 oxygen isotope ratio of -0.951 ‰ . PANWR is located within this area and depicted by the

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388 black triangle. [Solid lines are contours of predicted oxygen isotope ratios in barnacle calcite \(\$\delta^{18}\text{O}_c\$ \)](#).

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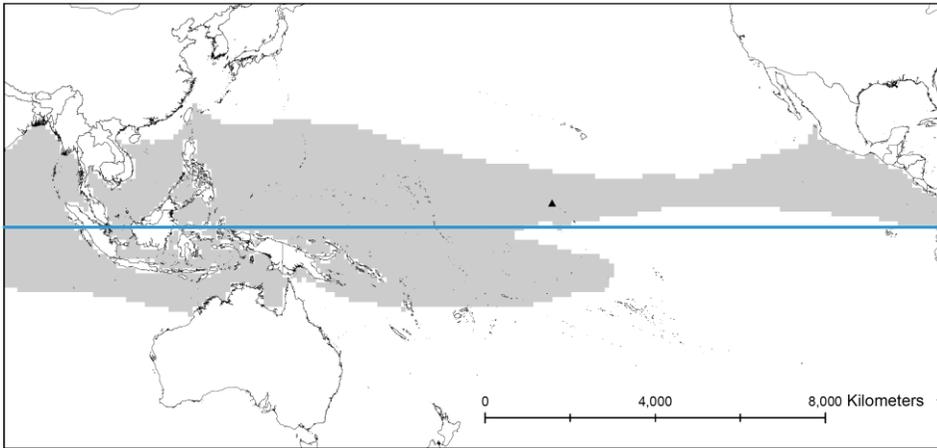
Fig. 2. Adjusted oxygen isoscape (shaded in gray). PANWR is depicted by the black triangle. Solid lines are contours of predicted oxygen isotope ratios in barnacle calcite ($\delta^{18}\text{O}_c$).

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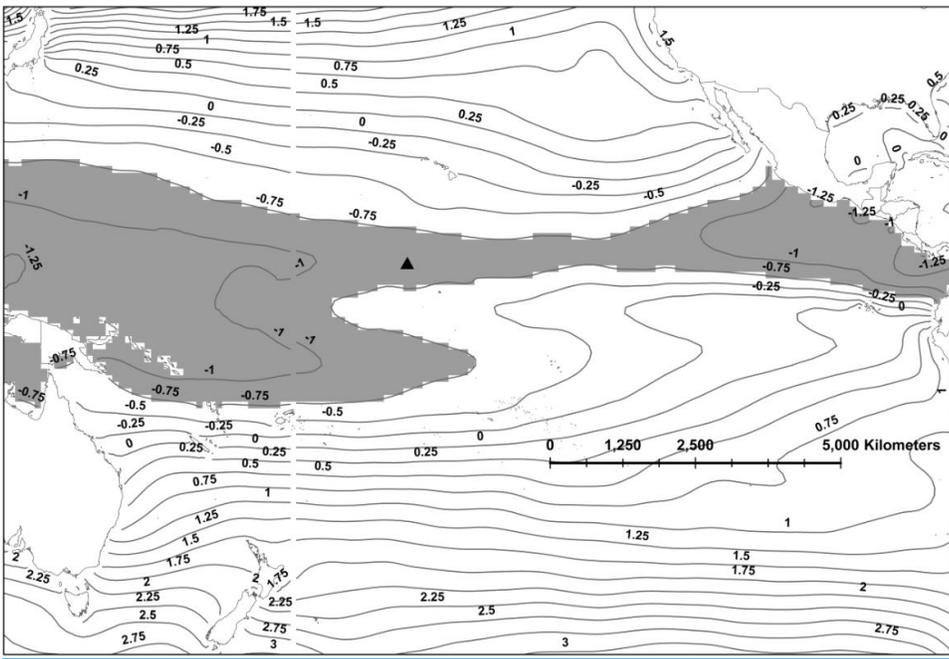
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