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1 **The use of stable isotope analyses from skin biopsy samples to assess trophic**
2 **relationships of sympatric delphinids off Moorea (French Polynesia)**

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24 **Abstract**

25 Defining trophic relationships among organisms of a community is critical in ecology. However,
26 the access to data is sometimes difficult, particularly in remote environments. Ecological niche
27 segregation among the most common delphinid species was investigated: the spinner dolphin
28 (*Stenella longirostris*), the roughed-toothed dolphin (*Steno bredanensis*), the short-finned pilot
29 whale (*Globicephala macrorhynchus*), and the melon-headed whale (*Peponocephala electra*).
30 Resource partitioning was explored by analysing $\delta^{13}\text{C}$ (reflecting foraging habitats) and $\delta^{15}\text{N}$
31 stable isotopes (reflecting trophic level) from skin biopsies collected around Moorea from July to
32 October 2002 to 2004. Results revealed that spinner dolphins had the lowest trophic level. The
33 three other species had similar $\delta^{15}\text{N}$ signatures. The most significant result is the differentiation
34 of *S. longirostris* from *S. bredanensis* and *G. macrorhynchus* but not from the *P. electra*. For the
35 latter three species, some degrees of overlap were apparent. For *S. longirostris*, *S. bredanensis*
36 and *G. macrorhynchus*, variation of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ stable isotope was not significant between
37 sexes. This study suggests that stable isotopes reveal some degree of segregation and overlap
38 within this delphinid community. However, fine-scale segregation processes may be concealed
39 by stable isotope analyses, meaning that traditional dietary analyses investigations are
40 complementary in answering questions related to niche segregation.

41
42 *Keywords:* delphinids, ecological niche, stable isotopes, carbon, nitrogen, biopsy samples,
43 Moorea, French Polynesia, South Pacific.

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48 **1. Introduction**

49 Sympatric species with similar ecological requirements can compete for resources and
50 thus their coexistence requires some degree of habitat and resource segregation (Pianka, 1974).
51 Indeed, similar species that co-occur are thought to compete for resources unless they occupy
52 different physical locations and/or feed on different prey. A shared resource in limited supply will
53 bring about competition between members of the same species (intra-specific competition) or
54 between individuals of different species (inter-specific competition) (Roughgarden, 1976).

55 Oceanic delphinids belong to 35 species worldwide (Jefferson *et al.*, 2008). Many of
56 them, have similar morphological characteristics, feeding habits and habitat preferences. This
57 phenomenon has been documented around tropical oceanic islands, where delphinid diversity and
58 biomass is generally high and where closely-related species co-occur (Gross *et al.*, 2009). Around
59 these islands, high cetacean diversity may be explained by the presence of a wide range of marine
60 habitats in close proximity to one another (Kiszka *et al.*, 2007). In addition, oceanic islands
61 appear to constitute areas of particular density of top predators due to an “island mass” effect.
62 Similar to continental margins, insular slopes of islands potentially provide more abundant
63 resources in the oligotrophic tropical marine environment (Guilmartin & Revelante, 1974). This
64 situation of sympatry suggests that fine-scale mechanisms allow for the partitioning of habitats
65 and/or resources. A study of the tropical delphinid community around the island of Mayotte, in
66 the Comoros Archipelago (south-western Indian Ocean), has shown that the ecological niches of
67 the delphinids occurring there do not overlap (Gross *et al.*, 2009). Indeed, these species capture
68 prey at different depths of the water column, where prey communities are segregated according to
69 species and size. In other areas, such as the Bahamas, the cetacean community shares habitat and
70 resources but only during the season when prey abundance is sufficient to support its needs,
71 while competitive exclusion exists for the rest of the year (MacLeod *et al.*, 2004). On the other

72 hand, top predators may overlap in their feeding habits due to low productivity of tropical waters
73 (Cherel *et al.*, 2008). If these shared resources are limited quantitatively, inter-species
74 competition can occur.

75 The dietary ecology of cetaceans and their trophic level can be determined using different
76 methods. The most extensively used consist in analysing the stomach contents of dead animals.
77 However, the specimens required for performing such analyses are often unavailable. The use of
78 naturally occurring nitrogen and carbon stable isotopes has provided alternative information from
79 which to better understand top predator feeding ecology, including marine mammals (Hobson &
80 Welch, 1992; Abend & Smith, 1995; Das *et al.*, 2003; Zhao *et al.*, 2004; Gross *et al.*, 2009). This
81 approach is generally considered as complementary to stomach content studies as it integrates
82 feeding habits on a longer-term basis. Various tissues, having varying temporal resolution
83 (turnover rates), may be used in stable isotope analyses, including skin (Gross *et al.*, 2009).
84 Turnover rate for this tissue has been estimated for the beluga whale (*Delphinapterus leucas*; St
85 Aubin *et al.*, 1990) and the common bottlenose dolphin (*Tursiops truncatus*; Hicks *et al.*, 1985).
86 The estimated time required for cell migration, from the basal lamina to the outermost surface, is
87 at least two months. The carbon and nitrogen isotope ratios ($^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$, expressed
88 hereafter as $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of a consumer reflect those of its diet, with a slight retention of the
89 heavier isotope and excretion of the lighter one (Das *et al.*, 2003). As a consequence, tissues will
90 be enriched with heavy isotopes at every trophic level (1‰ for $\delta^{13}\text{C}$ and 3‰ for $\delta^{15}\text{N}$). The minor
91 stepwise trophic enrichment of the carbon-isotope ratio limits its use in assessing trophic levels
92 but enhances its use in tracking carbon sources through a food chain. The carbon isotope ratio of
93 secondary and tertiary consumers should thus reflect the source of carbon at the base of their food
94 chain (Kelly, 2000).

95 Moorea, a volcanic tropical island in French Polynesia (South Pacific), is characterized by
96 the presence of many species of cetaceans, including several resident odontocetes, mostly
97 delphinids (Poole, 1993, 1995; Oremus *et al.*, 2007). At least thirteen species of dolphins may
98 coexist around the island. Of these, the most common are the spinner dolphin (*Stenella*
99 *longirostris*), the rough-toothed dolphin (*Steno bredanensis*), the short-finned pilot whale
100 (*Globicephala macrorhynchus*) and the melon-headed whale (*Peponocephala electra*) (Gannier,
101 2000). The present study aimed to investigate ecological niche partitioning in the dolphin
102 community of Moorea, especially for the spinner dolphin, the rough-toothed dolphin, the short-
103 finned pilot whale and the melon-headed whale. We concentrated on these four species as they
104 can be found within the same proximity around the island, in closely-related habitats within a
105 small area and at all seasons (Poole, 1993). We hypothesised that these four species have
106 different feeding niches that could be reflected in diverging stable isotope signatures. We also
107 investigated some potential segregation processes that may occur intra-specifically, especially
108 between sexes. Resource partitioning between sexes has been documented for a number of
109 species, including mammals such as the giraffe (*Giraffa camelopardalis*) and several primate
110 species (Beier, 1987; Young & Isbell, 1991). Sexual segregation in foraging habitats has also
111 been documented for some marine mammals, such as the grey seal (*Halichoerus grypus*) (Breed
112 *et al.*, 2006). Females may use higher quality food, especially during gestation and lactation;
113 therefore, it is often assumed that the energetic costs are greater for females than they are for
114 males (Key & Ross, 1999). This could result in diverging stable isotope signatures if females
115 develop sex-specific foraging strategies to fulfil their elevated energy requirements. On the other
116 hand, in dimorphic species, such as long-finned pilot whales (*Globicephala melas*), males seem
117 to have higher energetic needs (due to their larger size and weight) and potentially higher diving

118 capabilities, and consequently use larger and deeper-living prey than females (Desportes &
119 Mouritsen, 1993). As a consequence, males may have a higher trophic level than females.

120 In order to answer the question of niche segregation among the four most common
121 dolphin species around Moorea Island, and intra-specifically between sexes, we analysed $\delta^{13}\text{C}$
122 and $\delta^{15}\text{N}$ stable isotopes from skin biopsies collected from 2002 to 2004.

123

124 **2. Material and methods**

125 *2.1 Study area*

126 Moorea (17°30'S, 149°50'W) is a high volcanic island of the Society Archipelago (134
127 km²), French Polynesia, located in the central South Pacific (Figure 1). The island is almost
128 entirely surrounded by a barrier reef which delimits a lagoon system connected to the open ocean
129 by twelve passes varying in width and depth. Depth drops to more than 1000m just 2 to 3 km
130 outside the barrier reef. All species are usually observed outside the barrier reef, except the
131 spinner dolphin (*Stenella longirostris*) which commonly enters the lagoon through passes during
132 daytime (Poole, 1995) and feed in the open ocean only at night (Norris *et al.*, 1994).

133

134 *2.2 Sample collection*

135 Samples were collected from 2002 to 2004, during small-boat-based surveys (2002, n =
136 107; 2003, n = 32, 2004, n = 63), in sea conditions not exceeding Beaufort 3. Most of the
137 observation effort concentrated in austral winter (July – October). Efforts were made to survey
138 the entire coastline. However, the targeted species during these surveys were the spinner dolphin
139 and the humpback whale (*Megaptera novaeangliae*), and efforts were primarily concentrated in
140 nearshore waters (i.e., within 500 m from the barrier reef or within the lagoon), where these
141 species are preferentially distributed during daytime (Poole, 1995; 2002). Therefore, it must be

142 noted that search efforts were not optimal for encounters of more oceanic species. During each
143 encounter with dolphins, geographical position was recorded, group size was estimated by visual
144 counts, and photographs were taken using a digital camera equipped with a 70-300 mm lens. Skin
145 samples for genetic analyses were collected from adult dolphins using a small stainless-steel
146 biopsy dart fired from a modified veterinary capture rifle equipped with a variable pressure valve
147 (Krützen *et al.*, 2002). Behavioural responses to biopsy attempts were recorded and reported in
148 Oremus (2008). Level of short-term responses was low for all species and similar to that reported
149 elsewhere (e.g. Krützen *et al.*, 2002). All samples were preserved in 70% ethanol and stored at –
150 20°C for subsequent analysis.

151

152 2.3 Stable isotope analyses

153 Blubber and skin were separated for each biopsy. Stable isotope analyses were only
154 performed on the skin. The ethanol was evaporated at 45°C over 48 h and the samples were
155 ground and freeze-dried (Hobson *et al.*, 1997). The preservative used (ethanol) was the most
156 suitable that could be used due to logistical constraints. Ethanol storage may have variable and
157 organism-dependent effects on stable isotope signatures, generally higher on $\delta^{13}\text{C}$ values than on
158 $\delta^{15}\text{N}$ values (Kaehler & Pakhomov, 2001). It does not affect stable isotope signatures in
159 freshwater zooplankton and benthic macroinvertebrates (Siväranta *et al.*, 2008), bird eggs, blood
160 and muscle (Hobson *et al.*, 1997; Gloutney & Hobson, 1998). The increase in $\delta^{13}\text{C}$ values is
161 generally considered to be due to the extraction of some lipids but because lipids are depleted in
162 $\delta^{13}\text{C}$, they have anyway to be extracted to avoid a bias in the isotopic signature of $\delta^{13}\text{C}$ (De Niro
163 & Epstein, 1978; Tieszen *et al.*, 1983), that likely cancels any potential effect of storage in
164 ethanol. Lipid extraction was done by shaking (1 h at room temperature) in cyclohexane
165 (C_6H_{12}), and subsequent centrifugation prior to analysis. After drying, small sub-samples (0.35

166 to 0.45 mg + 0.001 mg) were prepared for analysis. Stable isotope measurements were performed
167 with a continuous-flow isotope-ratio mass spectrometer (Delta V Advantage, Thermo Scientific,
168 Germany) coupled to an elemental analyser (Flash EA1112 Thermo Scientific, Italy). Results are
169 expressed in δ notation relative to PeeDee Belemnite and atmospheric N₂ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$,
170 respectively, according to the equation

$$171 \quad \delta X = \left[\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right] \times 1000$$

172 where X is ^{13}C or ^{15}N and R is the isotope ratio $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$, respectively. Replicate
173 measurements of internal laboratory standards (acetanilide) indicated that measurement errors
174 were <0.1‰ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. Percent C and N elemental composition of tissues were obtained
175 using the elemental analyzer and used to calculate the sample C:N ratio, indicating good lipid
176 removal efficiency when <4.

177

178 *2.4 Species identification and molecular sexing*

179 Species sampled for this study were identified visually and confirmed using photographic
180 and genetic evidences. Mitochondrial DNA control region were sequenced for all samples, as
181 reported in Oremus *et al.* (2007), and sequences were submitted to the program DNA-
182 surveillance v. 3.01 (Ross *et al.*, 2003) to determine species identity. Sex was identified by co-
183 amplification of the male-specific *sry* gene and ZFX positive control gene (Gilson *et al.*, 1998).

184

185 *2.5 Data analysis*

186 Differences of stable isotopes signatures of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ among species were tested
187 using non-parametric Kruskal-Wallis tests. Pairwise tests to compare $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values
188 between species were performed using Mann-Whitney-*U* tests. Variability of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$

189 signatures among sexes was also tested using *U* tests. For all statistical analyses, a significance
190 level of $\alpha=0.05$ was used.

191

192 **3. Results**

193 *3.1 Sampling*

194 We collected skin samples from 91 delphinids: spinner dolphin ($N = 40$; 29 males and 11
195 females), rough-toothed dolphin ($N = 35$; 23 males and 12 females), short-finned pilot whale ($N =$
196 12; 6 males and 5 females) and melon-headed whale ($N = 4$; not sexed). All samples were
197 collected during the same season, i.e. austral winter (July to October). All sampled individuals
198 were considered to be adults based on their size.

199

200 *3.2 Resource partitioning*

201 The distribution of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values is given in Figure 2. Overall, overlap among
202 species is present, and intra-specific variability appears high. However, significant statistical
203 differences were found among the four species for $\delta^{15}\text{N}$ ($H = 24$; $df = 3$; $P < 0.001$) and $\delta^{13}\text{C}$
204 values ($H = 37$; $df = 3$; $P < 0.001$). Even with the melon-headed whale removed from the analysis,
205 differences remained significant (for $\delta^{15}\text{N}$: $H = 12.3$; $df = 2$; $P = 0.001$ and $\delta^{13}\text{C}$: $H = 13$; $df = 2$; P
206 $= 0.002$). Short-finned pilot whales had the highest $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ signatures, spinner dolphins
207 had the lowest $\delta^{15}\text{N}$ values (Figure 2), and melon-headed whales had the lowest $\delta^{13}\text{C}$ signatures.
208 Rough-toothed dolphins had an intermediate position between spinner dolphins and short-finned
209 pilot whales. At a finer scale, when looking at differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures between
210 species, some degrees of overlap and differentiation can be observed (Table 1). In $\delta^{13}\text{C}$,
211 differences were not significant between the spinner dolphin and the melon-headed whale nor

212 between the rough-toothed dolphin and the short-finned pilot whale. For other pairwise
213 comparisons of $\delta^{13}\text{C}$ values, statistically significant differences were found. For $\delta^{15}\text{N}$, overlap
214 was statistically significant between the melon-headed whale, the rough-toothed dolphin and the
215 short-finned pilot whale. The spinner dolphin could not be differentiated from the melon-headed
216 whale but differed significantly from the rough-toothed dolphin and short-finned pilot whale.

217

218 *3.3 Differences between sexes*

219 We tested differences in stable isotope signatures between males and females, both for
220 $\delta^{15}\text{N}$ and a $\delta^{13}\text{C}$, in all species except the melon-headed whale; in the latter case, sample size was
221 too small and sex data unavailable. Box plots (Figure 4) show stable isotope median values, 50,
222 75 percentiles and outliers of the three species. Male spinner dolphins and rough-toothed
223 dolphins seem to have a lower trophic position than females. In addition, both species seem to
224 feed on less ^{13}C -enriched preys (Figure 4). An opposite situation was found for short-finned pilot
225 whales, with males having higher mean $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ signatures. For the short-finned pilot
226 whale, males seem to have a wider range of $\delta^{13}\text{C}$ signatures (Figure 4). Conversely, females had a
227 wider range of $\delta^{15}\text{N}$ values. However, none of the differences were statistically significant
228 (spinner dolphins: $\delta^{15}\text{N}$: $U = 59$; $P = 0.236$; $\delta^{13}\text{C}$: $U = 33$; $P = 0.203$, rough-toothed dolphin:
229 $\delta^{15}\text{N}$: $U = 51$; $P = 0.266$; $\delta^{13}\text{C}$: $U = 57$; $P = 0.409$ and short-finned pilot whales: $\delta^{15}\text{N}$: $U = 50$; P
230 $= 0.193$; $\delta^{13}\text{C}$: $U = 13$; $P = 0.074$).

231

232 **4. Discussion**

233 *4.1 General comments*

234 Stable isotope approaches are powerful analytical tools to link the foraging ecology of top
235 predators with habitat, diving behaviour and diet (Das *et al.*, 2003; Zhao *et al.*, 2004). This has
236 been shown for a number of taxa, including seabirds (Cherel *et al.*, 2008), sharks (Comi *et al.*,
237 2005), pinnipeds (Zhao *et al.*, 2004) and cetaceans such as tropical delphinids (Gross *et al.*,
238 2009). These results were the first describing the isotopic niches of sympatric delphinids in
239 French Polynesia; they can be used in the future to examine seasonal, year-to-year or long term
240 variation in delphinid trophic ecology in the region. Lack of significance in some comparisons
241 may partly result from insufficient sample size. Analytical resolution was <0.1‰ as shown by
242 replicate measurements of internal laboratory standards, and delipidation was successfully carried
243 out on all samples since C:N ratios were always <4. Carbon sources and reference levels of
244 nitrogen were not investigated in this work; hence isotopic data can only be interpreted in terms
245 of relative values among the four species studied.

246

247 *4.2 Isotopic niche segregation*

248 Very few other studies have attempted to address issues regarding ecological segregation
249 in delphinid assemblages by analysing isotopic signature in skin biopsies (Gross *et al.*, 2009), but
250 more work has been done on communities of other marine top predators including sharks, large
251 teleost fishes, seabirds and marine mammals (Hobson & Welch, 1992; Abend & Smith, 1995;
252 Das *et al.*, 2003; Zhao *et al.*, 2004; Domi *et al.*, 2005; Ménard *et al.*, 2007; Cherel *et al.*, 2008;
253 Jaeger, 2009). In top predator communities, significant habitat partitioning has been found in
254 polar communities, such as in pinnipeds from the Antarctic (Zhao *et al.*, 2004). Conversely, in
255 tropical sympatric seabirds, significant overlap of feeding niches has been found at the
256 community level (Cherel *et al.*, 2008). This may be laid to the low productivity of tropical
257 oligotrophic waters, leading top predators to share the same feeding resources. However,

258 significant differences in isotopic niches were found in delphinids from the tropical island of
259 Mayotte, in the south-western Indian Ocean (Gross *et al.*, 2009). In the Southern Hemisphere,
260 clearly structured latitudinal carbon isoscapes have been found from the Antarctic to the
261 subtropical zones (Jaeger, 2009). This structured shape of latitudinal isoscapes may not exist in
262 tropical waters, mainly due to the oligotrophic nature of these waters. However, around oceanic
263 islands such as Mayotte, clear differences of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were observed between the
264 three delphinid genera investigated, which could be interpreted by the structured nature of marine
265 habitats (high carbon gradients from coastal to oceanic surface waters; Kiszka *et al.*, unpublished
266 data) around the island, from lagonal to oceanic waters. In addition, diving predators such as
267 small cetaceans use resources at varying depth, where carbon gradients are significant (from the
268 surface to bottom, where organic matter accumulates and provides carbon sources). In
269 conclusion, investigating trophic relationships of predators living in a structured system (such as
270 around an oceanic island) and feeding at varying depth may result in diverging isotopic niches,
271 while in surface feeders like seabirds, feeding in the homogenous oceanic system may result in
272 low to no difference in isotopic niches.

273 In our study, overall analyses show some degrees of niche partitioning among the four
274 species investigated, the most significant result being the differentiation in trophic level revealed
275 by $\delta^{15}\text{N}$ values among spinner dolphin, rough-toothed dolphin and short-finned pilot whale, but
276 not between spinner and melon-headed whale. In terms of foraging habitats, $\delta^{13}\text{C}$ values were
277 significantly different between all species except two pairs of species that could not be
278 discriminated: spinner dolphin/melon-headed whale and rough-toothed dolphin/short-finned pilot
279 whale. No significant intra-specific difference was found between sexes. Finally, it must be kept
280 in mind that differences in isotopic signatures are informative, whereas similarities do not
281 necessarily imply that species share a similar trophic niche; indeed different foraging strategies

282 may result in similar isotopic signatures. If stable isotope signatures and preys of two predators
283 are effectively similar, other segregation processes may occur, such as differential spatial and
284 temporal use of habitat and resources. Published studies from other areas in the Pacific suggest
285 that the community of delphinids around Moorea is likely to feed on pelagic and oceanic prey.
286 The spinner dolphin had the lowest trophic level and, with the melon-headed whale, the lowest
287 $\delta^{13}\text{C}$ values. The spinner dolphin feeds primarily on mesopelagic fishes and squids at night
288 (Norris *et al.*, 1994; Dolar *et al.*, 2003). Vertical distribution of the prey items summarized from
289 published literature indicate that spinner dolphins forage in the upper 200 meters and probably
290 occasionally as deep as 400 meters (Dolar *et al.*, 2003). Melon-headed whales are oceanic
291 predators, mostly feeding on mesopelagic fishes and cephalopods throughout their range
292 (Brownell *et al.*, 2009). When considering the vertical distribution of these prey groups, melon-
293 headed whales probably forage in the upper 700 meters (Young, 1978). Like spinner dolphins,
294 melon-headed whales seem to feed at night during vertical migrations of their preys, while they
295 rest and socialize during daytime near oceanic islands (Brownell *et al.*, 2009). Therefore, the two
296 species may share some similar features of habitat and resource use, although at Moorea, melon-
297 headed whales do not use inshore and nearshore waters like spinner dolphins do. This possible
298 overlap was confirmed in this study, as stable isotope signatures were not significantly
299 distinguishable. Note that this absence of significant difference could be due to small sample size
300 for melon-headed whales. However, around the island of Mayotte (with similar habitats to those
301 around Moorea) in the south-western Indian Ocean, stable isotope analyses on skin samples
302 revealed significant differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values with similar sample size than in our study
303 (Gross *et al.*, 2009). Melon-headed whales were characterised by significantly higher $\delta^{13}\text{C}$ and
304 $\delta^{15}\text{N}$ values (Gross *et al.*, 2009). In our study, $\delta^{13}\text{C}$ signatures were very similar between spinner
305 dolphins and melon-headed whales, suggesting they prey on species with similar $\delta^{13}\text{C}$ signatures

306 or even similar prey. This could suggest that their prey are not limited quantitatively, allowing
307 the two species to feed on similar resources without deleterious competition for one or the other.
308 However, despite no statistical evidences, some differences in the $\delta^{15}\text{N}$ values suggest that
309 melon-headed whale has a higher trophic level, and may probably feed on prey of higher trophic
310 position (probably larger prey). The feeding ecology of the rough-toothed dolphin is poorly
311 known, but it is known to feed on cephalopods (Aguiar dos Santos & Haimovici, 2001), deep
312 water fishes (Miyazaki & Perrin, 1994) and occasionally on coastal prey (Shallenberger, 1981).
313 Foraging on flying fishes and other surface fish has been regularly observed off Moorea (Oremus
314 & Poole, personal observations). In the eastern tropical Pacific, rough-toothed dolphins regularly
315 feed on dolphin fish (*Coryphaena hippurus*; Pitman & Stinchcomb, 2002). Short-finned pilot
316 whales are deep-water cephalopod predators (Hernandez-Garcia & Martin, 1994). Recent
317 evidence suggests that short-finned pilot whales can dive to depths reaching 1,000 meters on
318 occasion (Aguilar Soto *et al.*, 2008). Based on the available literature, making comparisons of the
319 diet of the rough-toothed dolphin and the short-finned pilot whale appears highly hazardous,
320 given the limited existing information. However, the latter seem to feed deeper in the water
321 column. In our results, the rough-toothed dolphin and the short-finned pilot whale were not
322 statistically different in their stable isotope signatures. However, *S. brenadensis* had lower $\delta^{13}\text{C}$
323 and $\delta^{15}\text{N}$ signatures than those in short-finned pilot whales. The later may feed on more carbon-
324 enriched prey. As there is a bottom-surface gradient of $\delta^{13}\text{C}$, with higher carbon values from the
325 sea bottom than at the surface (Hobson, 1999), higher $\delta^{13}\text{C}$ values observed in short-finned pilot
326 whales could be due to their preference for prey occurring deeper in the water column, in closer
327 proximity to bottom organic matter sources. This is consistent with published literature
328 describing general ecology, prey preferences and diving behaviour of short-finned pilot whales
329 (Aguilar Soto *et al.*, 2008; Hernandez-Garcia & Martin, 1994). The lower $\delta^{15}\text{N}$ values observed

330 in the rough-toothed dolphin may be attributed to its preference for preys of a lower trophic
331 position than those consumed by pilot whales.

332 Overall, when looking at biometrical data of predators from our study (Jefferson *et al.*,
333 2008), we observe that there is a correlation between trophic position and body size. Larger
334 predators have a higher trophic level in this delphinid community, which is consistent with many
335 other species communities.

336 Difference in feeding ecology between genders has been documented in delphinids
337 (Desportes & Mouritsen, 1993). From our dataset, we did not observed statistically significant
338 gender-specific variations of stable isotope signatures. However, mean values of carbon and
339 nitrogen isotope values were higher for female spinner and rough-toothed dolphins. On the
340 contrary, carbon and nitrogen isotope values were higher in male short-finned pilot whales. In
341 other words, the feeding niches of delphinids from Moorea may not differ according to sex.
342 However, some segregation processes may be not detected through the use of stable isotopes, and
343 traditional dietary analyses may answer this question. Detailed studies of diving behaviour may
344 also contribute to assessing gender-specific variation in feeding strategies. However, at least for
345 short-finned pilot whales, our sample size was small, which could conceal potential differences
346 related to sex. Indeed, it is known that males in sexually dimorphic species such as long-finned
347 pilot whales feed on larger prey, and potentially have a higher trophic level (Desportes &
348 Mouritsen, 1993). Nevertheless, no detailed studies of the diet of short-finned pilot whales have
349 been published, and such gender-specific variation may not occur in this species.

350 Overall, this study

351

352 **5. Conclusions**

353 When conventional dietary studies cannot be undertaken, such as around Moorea where
354 dead animals from strandings and bycatch are unavailable, the use of stable isotopes can be
355 recommended to assess trophic relationships in a community of cetacean predators, especially
356 delphinids. The delphinids around Moorea seem to have different feeding niches, although
357 statistical analyses do not always show significant differences among species. To complement the
358 interpretation of these first results, documenting accurate data on local carbon sources and
359 reference levels of nitrogen by analysing the isotopic content of particulate organic material that
360 constitute the basis of the local food webs and/or of a range of putative prey taxa from coastal to
361 oceanic habitats, would help in characterizing the three-dimensional isoscape (Jaeger, 2009) in
362 which this assemblage of sympatric dolphins dwells. Additionally, any information on the diet,
363 diving behaviour, activity budget and micro-scale spatial distribution of the four dolphin species
364 constituting this community would considerably improve the potential for interpreting stable
365 isotope data.

366

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374 **References**

375 Abend, A.G., Smith, T.D., 1995. Differences in ratios of stable isotopes of nitrogen in long-
376 finned pilot whales (*Globicephala melas*) in the western and eastern North Atlantic. ICES J. Mar.
377 Sci. 52, 837-841.

378 Aguiar dos Santos, R., Haimovici, M., 2001. Cephalopods in the diet of marine mammals
379 stranded or incidentally caught along southeastern and southern Brazil (21 – 34°S). Fish. Res. 52,
380 99-112.

381 Aguilar Soto, N., Johnson, M.P., Madsen, P.T., Díaz, F., Domínguez, I., Brito, A., Tyack, P.,
382 2008. Cheetahs of the deep sea: deep foraging sprints in short-finned pilot whales off Tenerife
383 (Canary Islands). J. Anim. Ecol., 77: 936-947.

384 Beier, P., 1987. Sex differences in quality of white-tailed deer diets. J. Mammal. 68, 323-329.

385 Breed, G.A., Bowen, W.D., McMillan, J.I. & Leonard, M.L., 2006. Sexual segregation of
386 seasonal foraging habitat in a non-migratory marine mammal. Proc. Royal Soc. B 273, 2319-
387 2326.

388 Brownell, R.L., Ralls, K., Baumann-Pickering, S., Poole, M.M., 2009. Behavior of melon-headed
389 whales, *Peponocephala electra*, near oceanic islands. Mar. Mam. Sci. 25, 639-658.

390 Cherel, Y., Le Corre, M., Jaquemet, S., Ménard, F., Richard, P., Weimerskirch, H., 2008.
391 Resource partitioning within a tropical seabird community: new information from stable isotopes.
392 Mar. Ecol. Prog. Ser. 366, 281-291.

393 Das, K., Lepoint, G., Leroy, Y., Bouquegneau, J.M., 2003. Marine mammals from the southern
394 North Sea: feeding ecology data from $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ measurements. Mar. Ecol. Prog. Ser. 263,
395 287-298.

396 De Niro, M.J., Epstein, S., 1978. Influence of diet on the distribution of carbon isotopes in
397 animals. Geo. Cosmo. Acta 42, 495-506.

398 Desportes, G., Mouritsen, R., 1993. Preliminary results on the diet of long-finned pilot whales off
399 the Faroe Islands. Rep.Int. Whal. Comm. (Special issue 14), 305-324.

400 Dolar, M.L.L., Walker, W.A., Kooyman, G.L., Perrin, W.F., 2003. Comparative feeding ecology
401 of spinner dolphins (*Stenella longirostris*) and Fraser's dolphins (*Lagenodelphis hosei*) in the
402 Sulu Sea. Mar. Mam. Sci. 19, 1-19.

403 Domi, N., Bouquegneau, J.-M., Das, K., 2005. Feeding ecology of five commercial shark species
404 of the Celtic Sea through stable isotope and trace metal analysis. Mar. Env. Res. 60, 551-569.

405 Gannier, A., 2000. Distribution of cetaceans off the Society Islands (French Polynesia) as
406 obtained from dedicated surveys. Aquat. Mamm. 26, 111-126.

407 Gilson, A., Syvanen, M., Levine, K., Banks, J., 1998. Deer gender determination by polymerase
408 chain reaction: validation study and application to tissues, bloodstains, and hair forensic samples
409 from California. Cal. Fish Game 84, 159-169.

410 Gloutney, M.L., Hobson, K.A., 1998. Field preservation techniques for the analysis of stable-
411 carbon and nitrogen isotopes in eggs. J. Field. Ornitho. 69, 223-237.

412 Gross, A., Kiszka, J., Van Canneyt, O., Richard, P., Ridoux, V., 2009. A preliminary study of
413 habitat and resource partitioning among co-occurring tropical dolphins around Mayotte,
414 southwest Indian Ocean. Estuar. Coast. Shelf. 84, 367-374.

415 Hernández-García, V., Martín, V., 1994. Cephalopods in the diet of two short-finned pilot whales
416 *Globicephala macrohynchus* Gray 1846 in the Canary Islands Area. International Council for the
417 Exploration of the sea. C.M. 1994/K: Shellfish Committee.

418 Hicks, B.D., St Aubin, D.J., Geraci, J.R., Brown, W.R., 1985. Epidermal growth in the bottlenose
419 dolphin, *Tursiops truncatus*. J. Invest. Dermatol. 85, 60-63.

420 Hobson, K.A., Welch, H.E., 1992. Determination of trophic relationships within a high Arctic
421 marine food web using $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analysis. *Mar. Ecol. Prog. Ser.* 84, 9-18.

422 Hobson, K.A., 1999. Tracing origins and migration of wildlife using stable isotopes: a review.
423 *Oecologia* 120, 314-326.

424 Hobson, K.A., Gibbs, H.L., Gloutney, M.L., 1997. Preservation of blood and tissue samples for
425 stable-carbon and stable-nitrogen isotope analysis. *Can. J. Zool.* 75, 1720-1723.

426 Jaeger, A., 2009. Etude isotopique des variations saisonnière et à long terme de l'écologie
427 alimentaire des oiseaux marins de l'océan Austral. PhD dissertation, University of Paris VI.

428 Jefferson, T.A., Fertl, D., Michael, M., Fagin, T.D., 2006. An unusual encounter with a mixed
429 school of melon-headed whales (*Peponocephala electra*) and rough-toothed dolphins (*Steno*
430 *bredanensis*) at Rota, northern Mariana Islands. *Micronesica* 38, 239-244.

431 Jefferson, T.A., Webber, A.M., Pitman, R.L., 2008. Marine mammals of the world. A
432 comprehensive guide to their identification. Elsevier, Oxford, United Kingdom.

433 Kaehler, S., Pakhomov, E.A., 2001. Effect of storage and preservation on the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$
434 signatures of selected marine organisms. *Mar. Ecol. Prog. Ser.* 219, 299-304.

435 Kelly, J.F., 2000. Stable isotopes of carbon and nitrogen in the study of avian and mammalian
436 trophic ecology. *Can. J. Zool.* 78, 1-27.

437 Key, C., Ross, C., 1999. Sex differences in energy expenditure in non-human primates. *Proc.*
438 *Royal Soc. B* 266, 2479-2485.

439 Kiszka, J., Ersts, P.J., Ridoux, V. 2007. Cetacean diversity around the Mozambique Channel
440 island of Mayotte (Comoros archipelago). *J. Cet. Res. Manag.* 9, 105-109.

441 Krützen M., Barré, L.M., Möller, L.M., Heithaus, M.R., Simmer, C., Sherwin, W.B., 2002. A
442 biopsy system for small cetaceans: darting success and wound healing in *Tursiops* spp. *Marine*
443 *Mar. Mam. Sci.* 18, 863-878.

444 MacLeod, C.D., Hauser, N., Peckman, H., 2004. Diversity, relative density and structure of the
445 cetacean community in summer months east of Great Abaco, Bahamas. J. Mar. Biol. Assoc. UK
446 84, 469-474.

447 Ménard, F., Lorrain, A., Potier, M., Marsac, F., 2007. Isotopic evidence of distinct feeding
448 ecologies and movement patterns in two migratory predators (yellowfin tuna and swordfish) in
449 the western Indian Ocean. Mar. Biol. 153, 141-152.

450 Miyazaki, N., Perrin, W.F., 1994. Rough-toothed dolphin *Steno bredanensis* (Lesson, 1828). Pp
451 1–21. In Ridgway, S. H. and R. J. Harrison, eds. Handbook of marine mammals. Vol. 5. The first
452 book of dolphins. Academic Press. London.

453 Norris, K.S., Dohl, T.P., 1980. Behavior of the Hawaiian spinner dolphin, *Stenella longirostris*.
454 Fish. Bull. 77: 821–849.

455 Norris, K.S., Würsig, B., Wells, R. S., Würsig, M., 1994. The Hawaiian Spinner Dolphin,
456 University of California Press, Berkeley, 408 pp.

457 Oremus, M., 2008. Genetic and demographic investigation of population structure and social
458 system in four delphinid species. PhD thesis, University of Auckland.

459 Oremus, M., Poole, M.M., Steel, D., Baker, C.S., 2007. Isolation and interchange among insular
460 spinner dolphin communities in the South Pacific revealed by individual identification and
461 genetic diversity. Mar. Ecol. Prog. Ser. 336, 275-289.

462 Pianka, E.R., 1974. Niche overlap and diffuse competition. Proc. Nat. Acad. Sci. USA 71, 2141-
463 2145.

464 Pitman, R.L., Stinchcomb, C., 2002. Rough-toothed dolphins (*Steno bredanensis*) as predators of
465 Mahimahi (*Coryphaena hippurus*). Pacific Science 56, 447-450.

466 Poole, M.M., 1993. A sighting and stranding network in French Polynesia, 1988-1993 Tenth
467 Biennial Conference on the Biology of Marine Mammals. Galveston, Texas.

468 Poole, M.M., 1995. Aspects of the behavioral ecology of spinner dolphins (*Stenella longirostris*)
469 in the nearshore waters of Mo'orea, French Polynesia. PhD thesis, University of California, Santa
470 Cruz.

471 Poole, M.M., 2002. Occurrence of humpback whales (*Megaptera novaeangliae*) in French
472 Polynesia in 1988-2001. Report SC/54/H14 to the Scientific Committee of the International
473 Whaling Commission.

474 Ross, H.A., Lento, G.M., Dalebout, M.L., Goode, M., Ewing, G., McLaren, P., Rodrigo, A.G.,
475 Lavery, S., Baker, C.S., 2003. DNA Surveillance: Web-based molecular identification of whales,
476 dolphins and porpoises. *J. Heredity*, 94: 111-114.

477 Roughgarden, J., 1976. Resource partitioning among competing species: a co-evolutionary
478 approach. *Theor. Pop. Biol.* 9, 388-424.

479 St Aubin, D.J., Smith, T.G., Geraci, J., 1990. Seasonal epidermal molt in beluga, *Delphinapterus*
480 *leucas*. *Can. J. Zool.* 68, 359-367.

481 Syväranta, J., Vesala, S., Rask, M., Ruuhijärvi, J., Jones, R.I., 2008. Evaluating the utility of
482 stable isotope analyses in archived freshwater sample materials. *Hydrobiologia* 600, 121-130.

483 Tieszen, L.L., Boutton, T.W., Tesdahl, K.G., Slade, N.A., 1983. Fractionation and turnover of
484 stable carbon isotopes in animal tissues: implications for $\delta^{13}\text{C}$ analysis of diet. *Oecologia* 57, 32-
485 37.

486 Young, R.E., 1978. Vertical distribution and photosensitive vesicles of pelagic cephalopods from
487 Hawaiian waters. *Fish. Bull.* 76, 583-615.

488 Young, T.P., Isbell, L.A., 1991. Sex differences in giraffe feeding ecology: energetic and social
489 constraints. *Ethol.* 87, 79-89.

490 Zhao, L., Castellini, M.A., Mau, T.L. & Trumble, S.J., 2004. Trophic interactions of Antarctic
491 seals as determined by stable isotope signatures. *Pol. Biol.* 27, 368-373.

492 Table 1: Pairwise Mann-Whitney U test p values for each pairs of delphinid species in carbon and
493 nitrogen. Values in bold are significant, meaning that the two species do not overlap in their
494 isotopic signatures. *Stenella longirostris*: spinner dolphin; *Peponocephala electra*: melon-headed
495 whale; *Steno bredanensis*: rough-toothed dolphin; *Globicephala macrorhynchus*: short-finned
496 pilot whale.

497

498 Figure 1: Location of the study area.

499

500 Figure 2: Stable isotope distribution ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in ‰) in delphinid skin tissues from Moorea.
501 *Stenella longirostris* (n = 40), *Steno bredanensis* (n = 35), *Globicephala macrorhynchus* (n = 12)
502 and *Peponocephala electra* (n = 4).

503

504 Figure 3: Stable isotopes ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in ‰) in delphinid skin tissues from Moorea. Graphs
505 show average values and standard deviations. *Stenella longirostris* (n = 40), *Steno bredanensis* (n
506 = 35) and *Globicephala macrorhynchus* (n = 12) and *Peponocephala electra* (n = 4).

507

508 Figure 4: Stable isotope ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in ‰) median values, 50, 75 percentiles and outliers of
509 *Stenella longirostris* (n = 40), *Steno bredanensis* (n = 35) and *Globicephala macrorhynchus* (n =
510 12) males versus females.

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$\delta^{13}\text{C}$	<i>Peponocephala electra</i>	<i>Globicephala macrorhynchus</i>	<i>Steno bredanensis</i>
<i>Stenella longirostris</i>	0.394	0.0001	0.0001
<i>Steno bredanensis</i>	0.005	0.131	
<i>Globicephala macrorhynchus</i>	0.006		
$\delta^{15}\text{N}$			
<i>Stenella longirostris</i>	0.09	0.00001	0.00004
<i>Steno bredanensis</i>	0.235	0.06	
<i>Globicephala macrorhynchus</i>	0.07		

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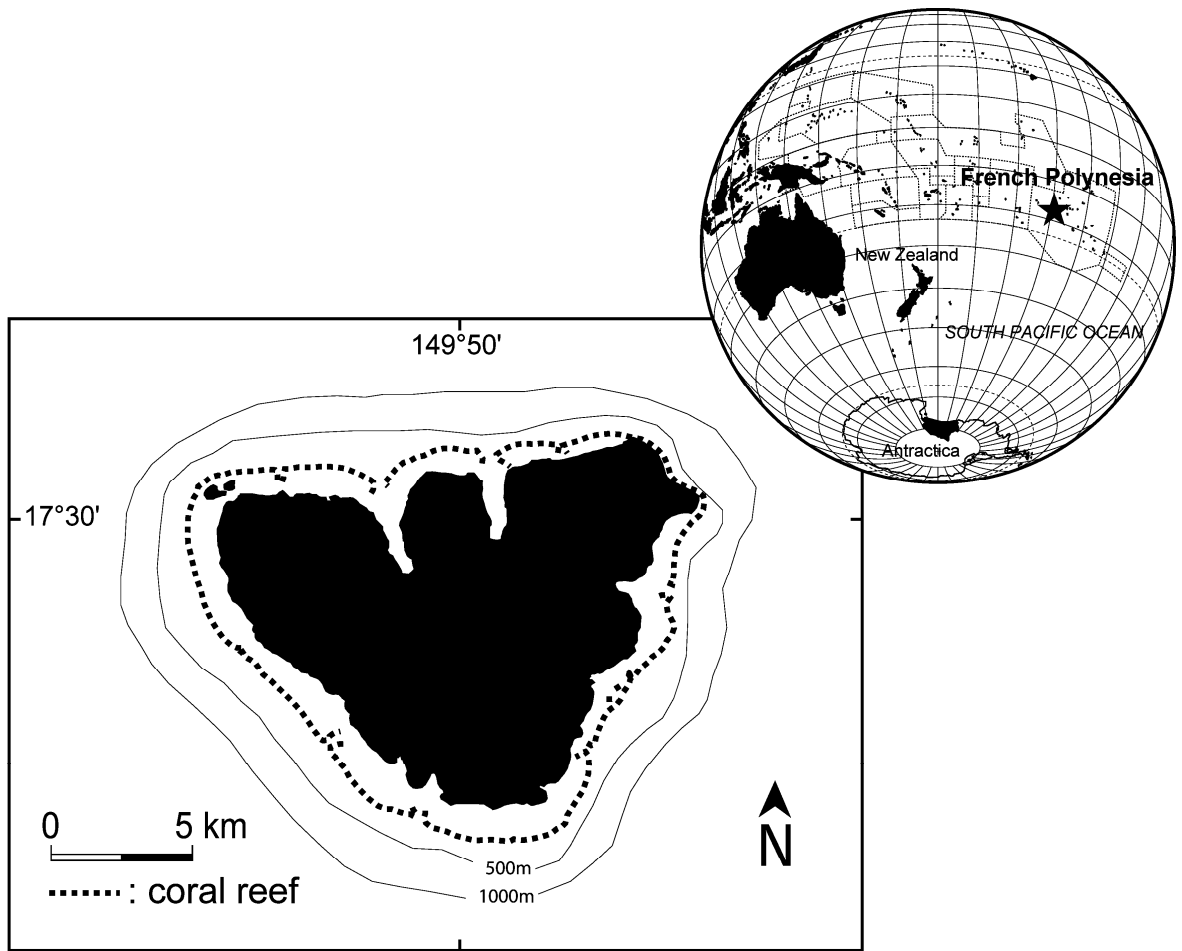
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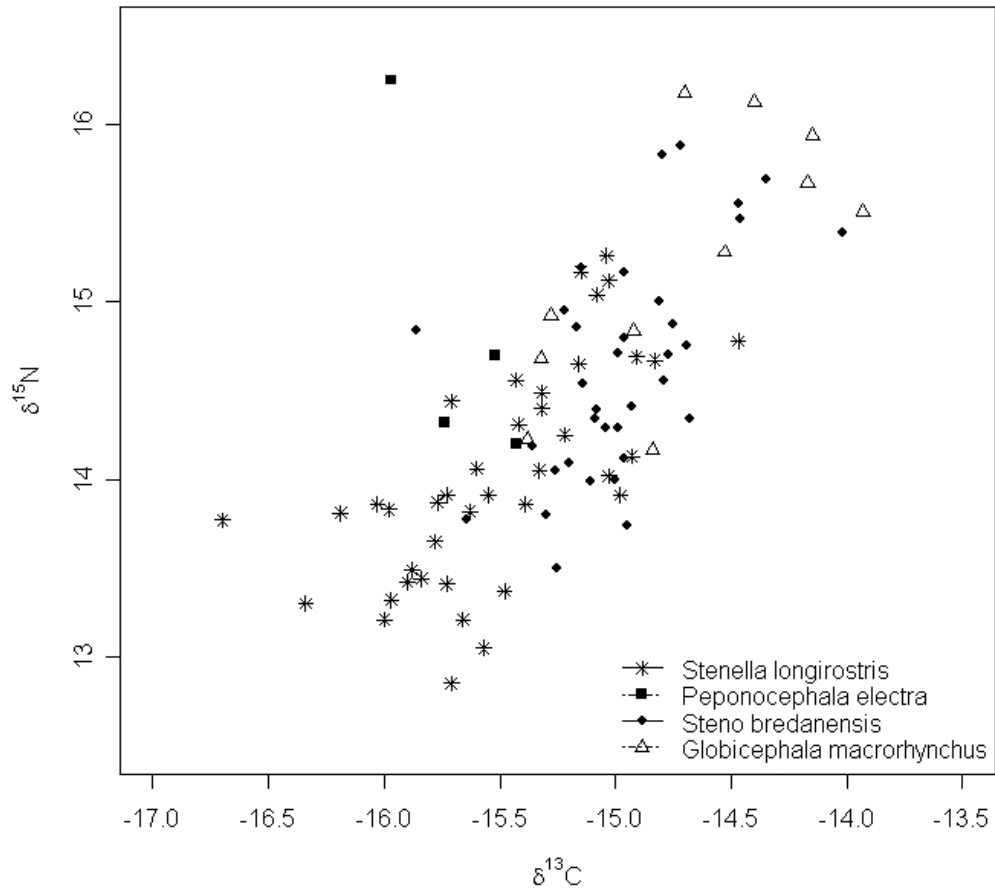
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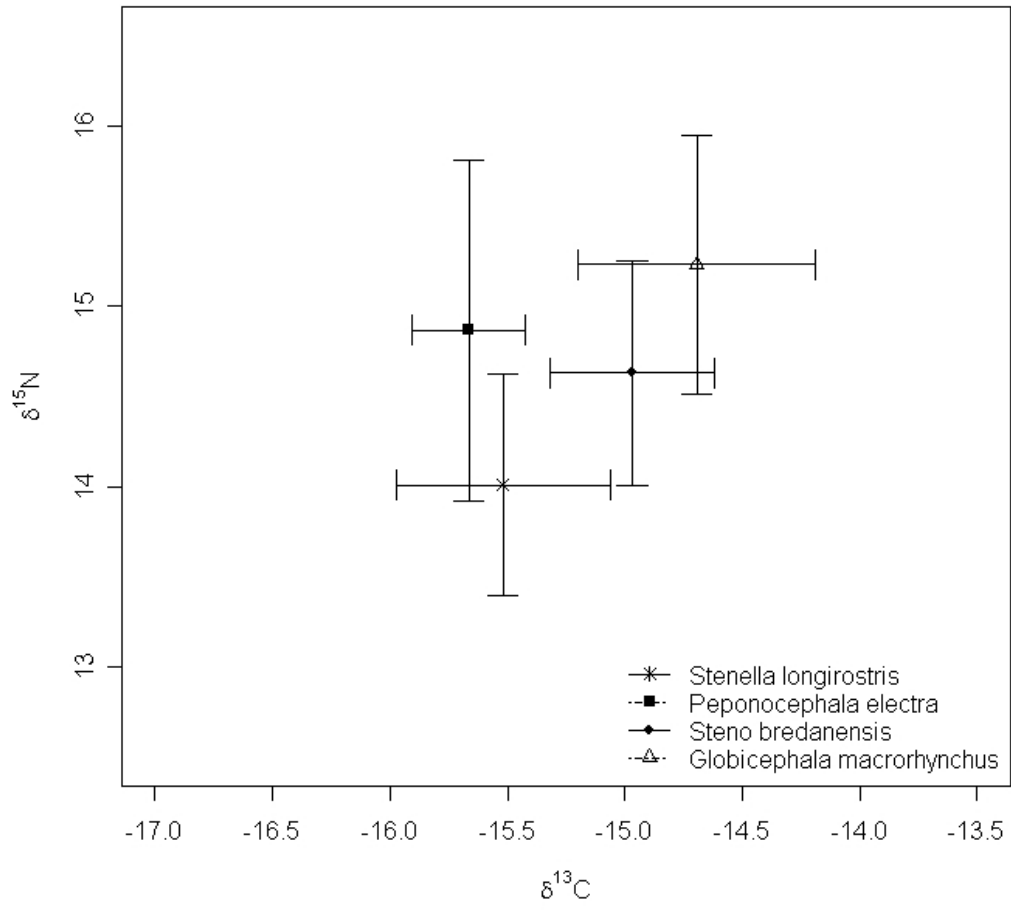


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$\delta^{13}\text{C}$

Stenella longirostris

$\delta^{15}\text{N}$

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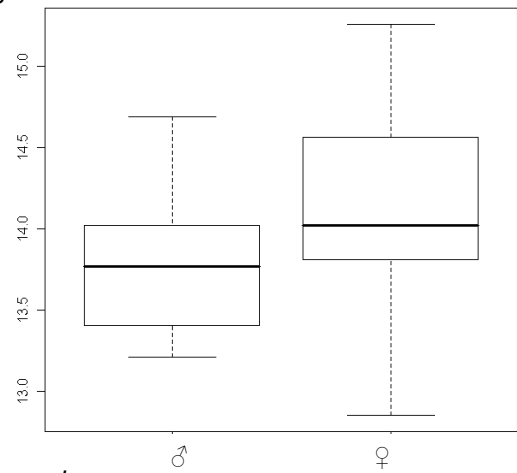
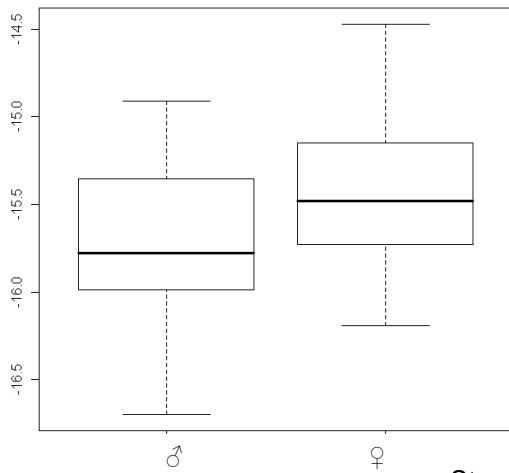
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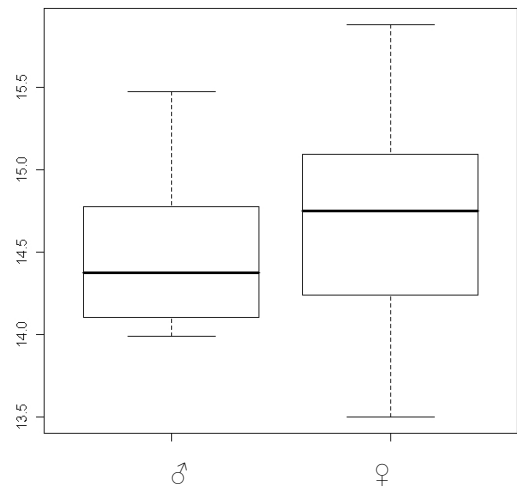
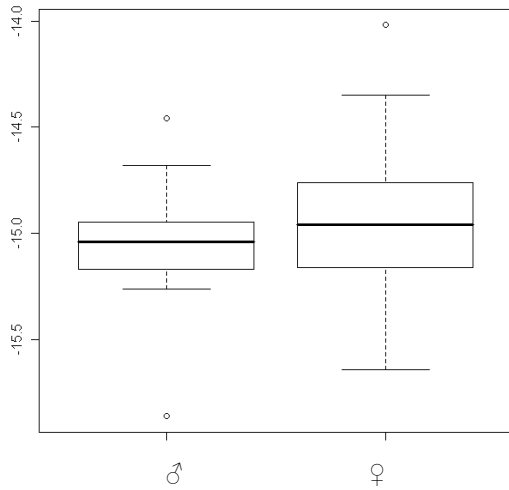
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Steno bredanensis



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Globicephala macrorhynchus

