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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 km<sup>2</sup>), 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 ( $\text{C}_6\text{H}_{12}$ ), 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  $\delta$  notation relative to PeeDee Belemnite and atmospheric N<sub>2</sub> for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ,  
170 respectively, according to the equation

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

172 where X is  $^{13}\text{C}$  or  $^{15}\text{N}$  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  $^{13}\text{C}$ -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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490 Zhao, L., Castellini, M.A., Mau, T.L. & Trumble, S.J., 2004. Trophic interactions of Antarctic  
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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	<b>0.0001</b>	<b>0.0001</b>
<i>Steno bredanensis</i>	<b>0.005</b>	0.131	
<i>Globicephala macrorhynchus</i>	<b>0.006</b>		
$\delta^{15}\text{N}$			
<i>Stenella longirostris</i>	0.09	<b>0.00001</b>	<b>0.00004</b>
<i>Steno bredanensis</i>	0.235	0.06	
<i>Globicephala macrorhynchus</i>	0.07		

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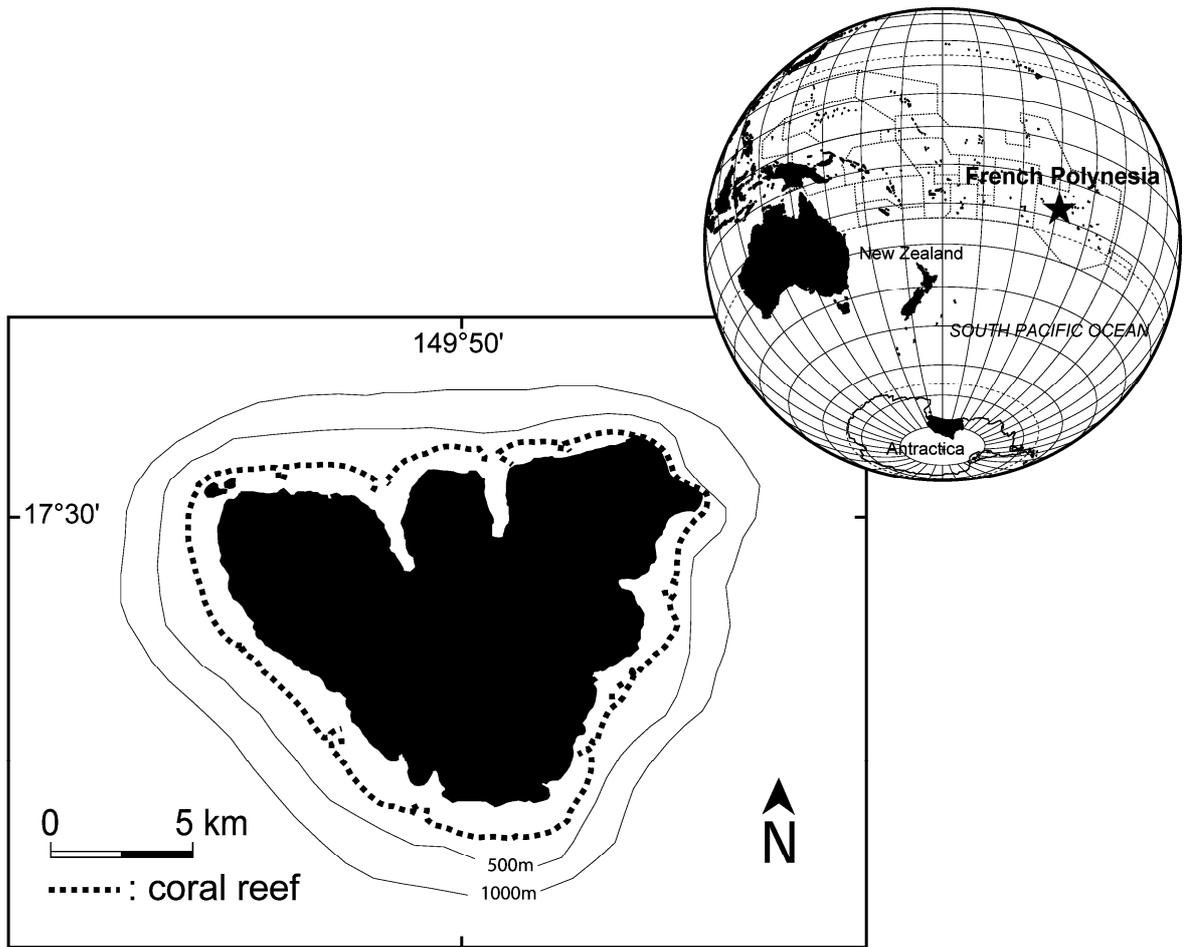
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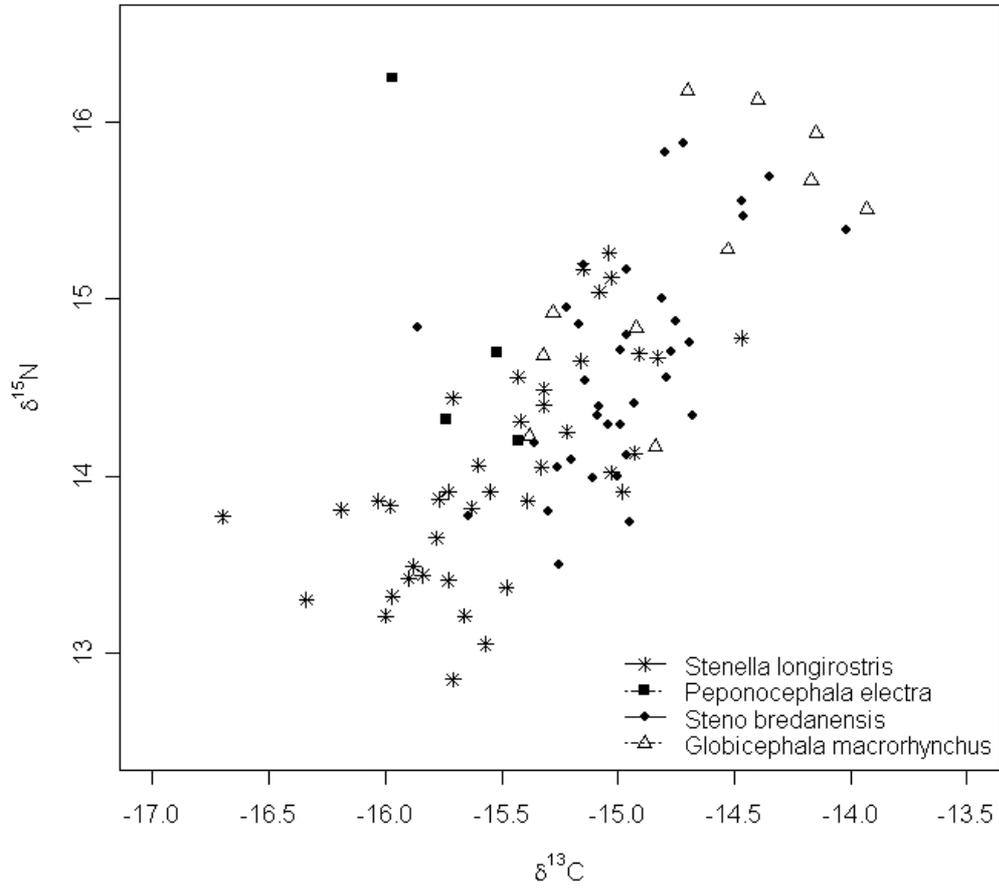
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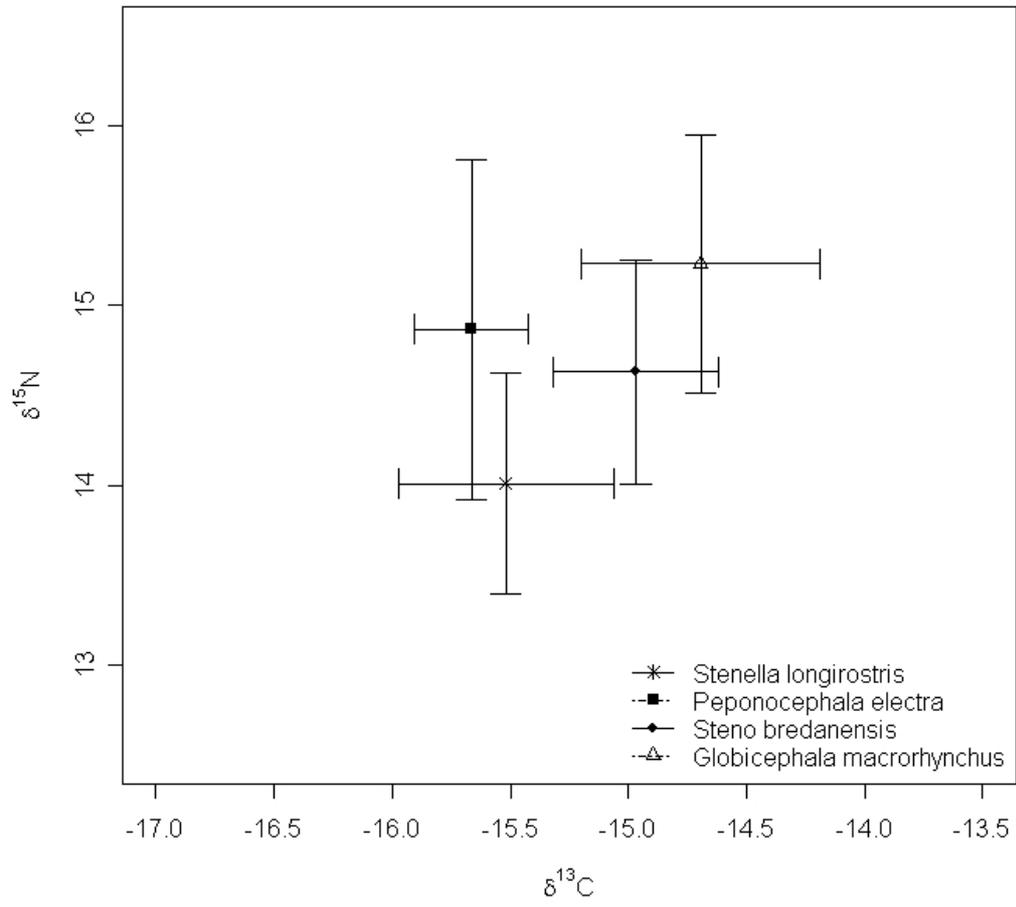


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