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1 **Tissue-specific fatty acid profiles of vent-obligate tonguefishes (*Symphurus* spp.) on**
2 **volcanic arcs in the western Pacific Ocean**

3

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23

24 **Abstract** Symphurine tonguefishes (Pleuronectiformes: Cynoglossidae) have been found at
25 hydrothermal vents on several submarine volcanoes in the western Pacific Ocean, often in great
26 numbers. Marine fish require dietary polyunsaturated fatty acids (PUFA) for growth and development,
27 but it is unclear how this need is met in environments based on chemosynthetic production.
28 Furthermore, the trophic ecology and nutritional requirements of symphurine tonguefishes at vents are
29 generally unknown. To address these knowledge gaps, fatty acid composition was determined in muscle,
30 liver, gonad and eyes of *Symphurus thermophilus* (Mariana Arc; 21-23°N, 142-144°E) and *Symphurus*
31 sp. A (Tonga Arc; 21°09' S, 175°45' W) sampled in October 2005 and May 2007, respectively. All
32 tissues of *Symphurus* spp. contained substantial levels of PUFA. Relative amounts of most PUFA in
33 *Symphurus* spp. muscle were similar to levels measured in non-vent demersal fish from temperate
34 waters worldwide. Principal components analysis (PCA) using *Symphurus* sp. A (Tonga Arc) fatty acid
35 profiles revealed three distinct clusters of samples that were related to tissue type. Similar trends could
36 not be discerned for *S. thermophilus* (Mariana Arc). The fatty acid composition of *Symphurus* spp.
37 suggests that PUFA-rich prey were not limiting at the sampled vents, although we lack information
38 about how these fish metabolize lipid. Stable carbon isotope ratios ($\delta^{13}\text{C}$) of several individual fatty
39 acids of fish from Nikko and Daikoku suggest a photic zone origin. Benthic-pelagic coupling on intra-
40 oceanic volcanic arcs may strongly influence the presence and persistence of resident vertebrates.

41

42

43 **Introduction**

44

45 Fish are common visitors at marine hydrothermal vents, but there are only seven known vent-obligate
46 species, from three genera: *Thermichthys hollisi* (Ophidiiformes: Bythitidae; Cohen et al. 1990),
47 *Thermarces cerberus*, *T. andersoni* (Perciformes: Zoarcidae; Rosenblatt and Cohen 1986), *T.*
48 *pelophilum* (Geistdoerfer 1999), *Symphurus thermophilus* (Pleuronectiformes: Cynoglossidae; Munroe
49 and Hashimoto 2008), *S. maculopinnis* (Munroe et al. 2011), and *Symphurus* sp. A (Tunnicliffe et al.
50 2010). It is difficult to observe and sample fish in these generally remote environments and little is
51 known about their trophic ecology. When samples can be obtained, analysis of lipid and stable isotope
52 biomarkers in fish tissues is highly informative and yields detailed dietary and nutritional information
53 (Guerreiro et al. 2004; Pond et al. 2008). Controlled experiments suggest that all marine fish require
54 'essential' polyunsaturated fatty acids (PUFA; 20:4 ω 6, 20:5 ω 3, 22:6 ω 3) in their diets to ensure proper

55 somatic and reproductive development and to maintain cell membrane integrity (Sargent et al. 1999).
56 These PUFA have important roles in vertebrate neural tissue and act as precursors in the formation of
57 eicosanoids, biologically-active signalling molecules (Ahlgren et al. 2009). At present almost nothing is
58 known about the fatty acid requirements of fish at hydrothermal vents as lipid composition has been
59 measured in a single vent-obligate species (Pond et al. 2008). Fatty acid compositional data could
60 inform our understanding of the trophic role of vent fish and their persistence in environments
61 dominated by chemosynthetic production, since the majority of PUFA found in marine settings are
62 thought to arise via photosynthesis (Kelly and Scheibling 2012).

63 Organic matter produced in the photic zone can reach deep hydrothermal vents (> 1500 m) but it
64 is not thought to make a significant contribution to local food webs (Levesque et al. 2005). The source
65 of the moderate PUFA levels in invertebrates from deep vents remains unclear, but may arise through
66 larval feeding and sequestration (Pond et al. 1997; Stevens et al. 2008), *in vivo* modification of dietary
67 fatty acids (discussed by Pond et al. 2002), and ingestion of PUFA-producing deep-sea microbes
68 (discussed by Stevens et al. 2015). Compound-specific stable isotope analysis of fatty acids in the vent-
69 obligate fish *T. cerberus* and its invertebrate prey from deep sites (> 2500 m) on the East Pacific Rise
70 indicated that 20:5 ω 3 originated in the vent environment, while the source of 22:6 ω 3 was planktonic
71 (Pond et al. 2008). The lipid composition of *T. cerberus* differed from the majority of demersal teleosts
72 because the dominant PUFA was 20:5 ω 3, and not 22:6 ω 3, and relative amounts of PUFA in *T. cerberus*
73 were generally much lower (Bakes et al. 1995; Budge et al. 2002; Økland et al. 2005; Stowasser et al.
74 2009). Fatty acid levels have not been measured in the tissues of other vent-obligate fish species,
75 including the symphurine tonguefishes found at relatively shallow vents (i.e., ~80 - 730 m) on volcanic
76 arcs in the western Pacific Ocean (Munroe and Hashimoto 2008; Tunnicliffe et al. 2010; 2013). In these
77 shallow ecosystems, PUFA are incorporated into the tissues of resident invertebrate vent obligates via
78 direct grazing on adjacent photosynthetic primary producers or through ingestion of particles that
79 originated in the photic zone (Comeault et al. 2010; Stevens et al. 2015). It is therefore probable that fish
80 at shallow oceanic hydrothermal vents also have greater access to PUFA, compared to those at deep
81 vents, and that this enhanced supply would be reflected in their lipid composition.

82 Symphurine tonguefishes (*Symphurus* spp.) have been found at hydrothermal vents in the
83 Okinawa Trough and on submarine volcanoes along the Izu-Bonin-Mariana Arc and the Tonga-
84 Kermadec Arc (Munroe and Hashimoto 2008; Tunnicliffe et al. 2010; 2013). These fish reside on a
85 number of different substrata and at some locations they form dense aggregations; on submarine volcano

86 Daikoku (Mariana Arc) maximum densities can exceed 240 individuals m⁻² (Tunnicliffe et al. 2013).
87 Observations of *Symphurus* spp. gut contents at western Pacific vents (Tunnicliffe et al. 2010; 2013)
88 revealed mostly remains of crustaceans and polychaetes, but these studies did not focus on trophic
89 ecology. Furthermore, fatty acid composition integrates dietary signals over a longer time frame
90 (Iverson et al. 2004) and thus alleviates some of the caveats associated with gut content analysis in fish
91 (Reñones et al. 2002). Pleuronectid flatfish require high levels of dietary PUFA, 22:6 ω 3 in particular
92 (Copeman et al. 2002). Insufficient levels of this nutrient can affect foraging efficiency, growth and
93 survival of teleosts (Sargent et al. 1997) and can impair metamorphosis in pleuronectids (Hamre et al.
94 2007). Measurements of PUFA levels in *Symphurus* spp. are therefore of primary importance in
95 understanding their presence and role in hydrothermal vent food webs.

96 In this paper we report the first fatty acid compositions of the vent-obligate tonguefishes *S.*
97 *thermophilus* and *Symphurus* sp. A collected from hydrothermal vents on submarine volcanoes of the
98 Mariana and Tonga Arcs, respectively. We determined fatty acid composition in muscle, liver, gonad
99 and eyes of *Symphurus* spp. because investigating fatty acid profiles in different body tissues can help
100 discern nutritional and physiological adaptations of deep-sea fish (Drazen et al. 2009; Stowasser et al.
101 2009; Mayor et al. 2013). Based on previous work at shallow vents (e.g., Tunnicliffe et al. 2013; Stevens
102 et al. 2015), we made the following predictions: 1. Given the observed dense aggregations of *Symphurus*
103 spp. and the presence of PUFA-rich prey in their diets (e.g., crustaceans), we expect that their tissues
104 will contain substantial levels of PUFA, and therefore, 2. *Symphurus* spp. fatty acid composition will be
105 more similar to demersal fish from PUFA-rich ecosystems, than to the deep-water, vent-obligate species
106 *Thermarces cerberus*.

107
108

109 **Materials and Methods**

110

111 Field sampling and site characteristics - Mariana Arc

112

113 During October 2005, four individuals of *Symphurus thermophilus* were collected using the suction
114 sampler on the Remotely Operated Vehicle (ROV) *Hyper Dolphin*, during a cruise aboard the R/V
115 *Natsushima*. Two fish each were obtained from the submarine volcanoes Nikko and Daikoku at depths
116 of 459 and 383 m, respectively (Table 1). The physical setting, geological and hydrothermal features,

117 and associated biological communities on Nikko and Daikoku are described in detail in Tunnicliffe et al.
118 (2013), but we include a brief summary below.

119 On Nikko, hydrothermal venting was concentrated in and around the crater on the western
120 summit cone, at a depth of ~400 m. The crater was composed of andesite outcrops covered in thick
121 sulphur crusts. Pools of liquid sulphur associated with high temperature venting were observed. The
122 walls and floor of the summit crater supported a large and diverse hydrothermal community including *S.*
123 *thermophilus*, vestimentiferan tubeworms, alvinocaridid shrimp, palaemonid shrimp, bythograeid crabs
124 and branchiopolynoid scale worms. *Symphurus thermophilus* was observed between 380 and 475 m.
125 Hydrothermal venting occurred on the slopes of Daikoku seamount toward the summit of its cone. The
126 slope was made up of sulphur flows and detrital sulphur sediments. Molten sulphur pools were also
127 present. *Symphurus thermophilus* was found at depths of 374 to 440 m; other members of the biological
128 community were mangeliid gastropods, paguroid crabs, bythograeid crabs and barnacles. *Symphurus*
129 *thermophilus* was found on a variety of substrates including volcanoclastic and sulphur sediments
130 (Nikko and Daikoku), among tubeworm bushes (Nikko), and on sulphur crusts near molten sulphur and
131 high-temperature vents (Nikko and Daikoku). *Symphurus thermophilus* occurred in low abundance in
132 areas with dense microbial mats and on rock surfaces beyond the influence of hydrothermal venting.

133

134 Field sampling and site characteristics - Tonga Arc

135

136 Five specimens of the cryptic species *Symphurus* sp. A were collected at Volcano-1 during a cruise
137 aboard the R/V *Sonne* in May 2007 (Table 1). Fish traps baited with catfood were deployed at a depth of
138 198 m and retrieved using the ROV ROPOS (site *Fish Traps*). Tunnicliffe et al. (2010) described the
139 sampling location and fish habitat. To summarize, lavas on Volcano-1 were composed of basaltic
140 andesite and thick beds of cemented ash. *Symphurus* sp. A was observed between depths of 83 and 288
141 m among bathymodiolid mussel beds, and on sedimented substrata, yellow sulphur flows and vertical
142 walls and chimneys. *Symphurus* sp. A was closely associated with hydrothermalism and abundances
143 were low on surfaces colonized by microbial mats. At *Fish Traps*, brachyuran crabs were also abundant.

144

145 Lipid analysis

146

147 Frozen fish were thawed, weighed, measured (total length) and individual tissues dissected. Skin was
148 removed from the left side of the fish where muscle and gonad samples were taken. The operculum was
149 removed and the liver and gut were excised. Livers were not sampled in samples N1 and D1. In fish
150 from Volcano-1, gut contents were dissected and analyzed separately from the gut tissue. In all fish, the
151 eyes were also removed for analysis. All tissues were lyophilized for 24 h and the dry mass measured.
152 Dried tissues were homogenized in 2:1 chloroform:methanol and extracted according to Folch et al.
153 (1957). Total lipid in muscle was determined gravimetrically. Lipid extracts were derivatized with an
154 internal standard (23:0) using methanol and sulphuric acid (Christie 2003) and the resulting fatty acid
155 methyl esters (FAME) were purified by thin-layer chromatography (Pond et al. 2008). Purified FAME
156 were analyzed using a Thermo Finnigan GC equipped with a Restek Stabilwax column (30 m x 0.25 mm
157 x 0.25 μm) using hydrogen as the carrier gas. Fatty acid identification was performed using commercial
158 standards (e.g., Marinol) and via picolinyl esters (Christie 2003), which were analysed on a Thermo
159 Finnigan GC/MS equipped with the same type of column as the GC. To enable comparison with
160 literature values, muscle dry mass was converted to wet mass by assuming a 72% water content (Holmes
161 and Donaldson 1969); this allowed us to express total lipid and total PUFA per unit wet muscle mass.
162 We define PUFA (polyunsaturated fatty acids) as fatty acids containing 3 or more double bonds.

163

164 Compound-specific stable isotope analysis

165

166 Carbon stable isotope ratios ($\delta^{13}\text{C}\%$ Vienna Pee-Dee Belemnite) in tissues of *S. thermophilus* from the
167 Mariana Arc were measured by GC-IRMS using a VG Isochrom II instrument as detailed in Pond et al.
168 (2008). Isotope ratios were analysed separately in samples of gut and muscle tissue from fish N1, N2,
169 D1 and D2 (Table 1); all carbon stable isotope data from Nikko and Daikoku were then combined and
170 averaged separately.

171

172 Principal components analysis

173

174 To discern differences in lipid composition between *Symphurus* spp. tissues, principal components
175 analysis (PCA) was performed using fatty acid percentage data, total PUFA (mg g^{-1} dry mass), fish total
176 length (cm), and fish mass (g). The Mariana Arc samples (*S. thermophilus*) were analysed separately
177 from those collected on the Tonga Arc (*Symphurus* sp. A), since some differences in fatty acid

178 composition of fish were noted between the two locations. All observations were normalized by
179 conversion to z-scores prior to analysis (Legendre and Legendre 2012). Only variables with correlation
180 coefficients ≥ 0.7 were included on Figs. 1 and 2 (Meglen 1992). PCA clusters were determined via
181 hierarchical cluster analysis on PC1 and PC2 scores. All analyses were performed with Statistica 6.0.

182

183

184 **Results**

185

186 Physical characteristics of fish

187

188 Six female and three male fish were collected, and most had empty guts (Table 1). On Volcano-1, two
189 fish had visibly distended guts and an intact polychaete and crustacean remains were recovered from
190 two separate fish. Fish from Nikko were usually longer and heavier than those from Daikoku and
191 Volcano-1.

192

193 Lipid composition of fish tissues and gut contents

194

195 Total lipid in fish muscle ranged from 8.8 to 17.5 mg g⁻¹ wet mass, with the highest values found in the
196 Mariana Arc samples (Table 2). PUFA levels in muscle were between ~2.0 and 2.5 mg g⁻¹ wet mass
197 except for sample D1 from Daikoku, where they were very low (Table 2). At shallow hydrothermal
198 vents, 20- and 22-carbon PUFA are thought to originate in the photic zone, while 16:1 ω 7, 18:1 ω 7,
199 18:2 ω 6, NMID (non-methylene interrupted dienes), and 20:1 ω 7 can be indicative of bacteria (Pond et al.
200 1998; Stevens et al. 2015). Muscle, gonad, liver and eyes in *Symphurus* spp. were dominated by the
201 same fatty acids: 16:0, 16:1 ω 7, 18:0, 18:1 ω 9, 18:1 ω 7, 20:4 ω 6, 20:5 ω 3, 22:5 ω 3 and 22:6 ω 3 (Tables 2-5).
202 In terms of PUFA, 22:6 ω 3 was the dominant fatty acid in all tissues and the 22:6 ω 3:20:5 ω 3 ratio was
203 almost always ≥ 1 . Muscle fatty acid composition was relatively constant between samples, although
204 fish D1 was markedly different. Sample D1 was characterized by much lower relative amounts of PUFA
205 and higher SFA (saturated fatty acids) and MUFA (monounsaturated fatty acids) levels, particularly
206 16:0, 18:0, 16:1 ω 7 and 18:1 ω 7. Gonads in tonguefishes from the Mariana Arc, in particular those from
207 fish N1, N2 and D1, were poor in several PUFA, including 20:4 ω 6, 20:5 ω 3 and 22:5 ω 3, as compared to
208 all other samples. No sex-based patterns in fatty acid composition were discerned. Although we have an

209 incomplete data set, liver tissue was composed predominantly of SFA and MUFA, with the lowest
210 PUFA levels found in sample D2. The fatty acid composition of the eyes was variable across samples;
211 22:6 ω 3 was present in very high amounts in several fish, particularly those from Volcano-1, while
212 relative amounts of this and other PUFA (e.g., 20:4 ω 6, 20:5 ω 3, 22:5 ω 3) were lower in samples N2, D1
213 and D2.

214 An unidentified crustacean and a polychaete recovered from the guts of *Symphurus* sp. A on
215 Volcano-1 had dissimilar fatty acid compositions (data not shown). The crustacean was rich in PUFA
216 (40% of total fatty acids), principally 20:5 ω 3 (10.1%) and 22:6 ω 3 (18.5%), and was characterized by
217 moderate 16:1 ω 7 (6.8%), 18:1 ω 7 (6.4%) and 18:1 ω 9 (9.1%) levels. This combination of fatty acids, in
218 particular 18:1 ω 9, 20:5 ω 3 and 22:6 ω 3 together, is typical of crustaceans (Parrish et al. 2009). Although
219 the polychaete was less rich in Σ PUFA (30.2%), and contained very little 22:6 ω 3 (0.5%), a large relative
220 amount of 20:5 ω 3 (16.5%) was present. In terms of MUFA, the polychaete was characterized by
221 relatively elevated levels of 17:1 (1 isomer; 7.1%), 20:1 (4 isomers; 10.3%), and 22:1 ω 7 (6.6%). This
222 fatty acid composition is intermediate between that of polychaetes at deep hydrothermal vents (Phleger
223 et al. 2005) and those in cold coastal benthos (Parrish et al. 2009).

224 Principal component 1 (PC1) explained 52% of the variability among samples of *Symphurus*
225 *thermophilus* from the Mariana Arc, and divided them according to fish size and degree of fatty acid
226 unsaturation (Fig. 1). Specifically, gonad, muscle and eyes from small-sized, low-mass fish D1 formed a
227 distinct cluster characterized by low levels of PUFA. A small cluster (-PC1) consisting of two muscle
228 and one eye sample was separated from all others due to high relative amounts of total PUFA, 20:4 ω 6,
229 20:5 ω 3 and 22:6 ω 3. A large number of samples, mostly gonad, eye and liver tissues from both Nikko
230 and Daikoku, formed a central cluster and had mixed PUFA/MUFA fatty acid compositions. At
231 Volcano-1, three clusters of samples from *Symphurus* sp. A were distinguished by the PCA, one of
232 which was composed solely of eye samples (+PC1) and was characterized by a unique combination of
233 18:0 and several PUFA (Fig. 2). The cluster in negative PC1 space contained samples from four livers
234 and one gonad, all of which were composed of a mixture of MUFA and PUFA. The gonad and muscle
235 samples in the large cluster centred on PC1 also contained a mixture of PUFA and some MUFA,
236 including biomarkers associated with both crustaceans and polychaetes (e.g., 22:1 ω 7, 20:5 ω 3, 22:6 ω 3).

237

238 Stable carbon isotope composition of individual fatty acids

239

240 Fatty acids in *S. thermophilus* from Nikko were generally depleted in ^{13}C compared to those in fish from
241 Daikoku, although $\delta^{13}\text{C}$ ratios of 22:6 ω 3 were essentially the same ($\sim -23\%$) in samples of fish from
242 both submarine volcanoes (Table 6). Descending particles that originate via photosynthesis in the photic
243 zone are generally characterized by $\delta^{13}\text{C}$ ratios of $\sim -22\%$ (Gebruk et al. 2000), which overlaps with
244 stable carbon isotope values for most of the fatty acids from fish at Nikko (all except 20:4 ω 6 and
245 20:5 ω 3) and approximately half of the fatty acids in fish from Daikoku.

246

247

248 **Discussion**

249

250 Differences in the fatty acid composition of individual tissues were clearly seen in *Symphurus* sp. A
251 from Volcano-1 (Tonga Arc). Eye tissue from *Symphurus* sp. A was distinct from all other samples
252 based on its fatty acid profile, specifically the combination of relatively high levels of several PUFA and
253 18:0. In particular, the fatty acid 22:6 ω 3 was abundant in the eyes of *Symphurus* sp. A, at levels similar
254 to those of non-vent flatfish (Estévez and Kanazawa 1996; Shields et al. 1999). Fatty acids have not
255 been analysed in the eyes of other vent-obligate fish. In marine fish tissues the presence of 22:6 ω 3 is
256 thought to be solely dietary since, unlike many freshwater species, they lack the enzymes necessary to
257 elongate and desaturate fatty acids to produce long-chain PUFA (Saito et al. 1997; Tocher 2003). The
258 mean stable carbon isotope signature of 22:6 ω 3 in the tissues of vent-obligate fish at both deep (mean
259 $\delta^{13}\text{C}=-22.7\%$; Pond et al. 2008) and shallow sites (mean $\delta^{13}\text{C}=-23.2\% \pm 3.8$, $n=16$; Table 6) corroborate
260 this assertion and indicate a planktonic origin for this fatty acid. Phytoplankton is the principal source of
261 22:6 ω 3 in the marine environment (Kelly and Scheibling 2012) since macroalgae do not produce it in
262 significant quantities (Pereira et al. 2012) and only a few strains of deep-sea bacteria capable of
263 producing 22:6 ω 3 have been identified (El Razak et al. 2014). Because dietary 22:6 ω 3 subsequently
264 affects levels in neural tissue (Bell et al. 1995; Sargent et al. 1997; Bell et al. 2003), we suggest that the
265 levels of this fatty acid in the eye tissue of *Symphurus* sp. A indicate that it was not limiting at shallow
266 hydrothermal vents on the Tonga Arc. However, the specific nutritional requirements (e.g., essential
267 fatty acids) of *Symphurus* spp. and the degree to which they modify and sequester dietary fatty acids are
268 unknown.

269 The livers of *Symphurus* sp. A were characterized by comparatively higher levels of MUFA and
270 SFA, in particular 18:1 ω 7 and 16:0, although the PCA did not cluster all liver samples together. The

271 liver is an important site of lipid storage in many fish (Sargent 1995; Tocher 2003) and its fatty acid
272 composition closely mirrors diet in flatfish (Dwyer et al. 2003). Many deep-sea fish also have high
273 MUFA levels in the liver, including the vent-obligate *T. cerberus* (Pond et al. 2008), several species of
274 hydrothermal vent visitors (Guerreiro et al. 2004), and some abyssal macrourids (Stowasser et al. 2009).
275 However, MUFA levels in *Symphurus* sp. A liver were generally lower (and thus Σ PUFA higher) than in
276 these deep-sea fish listed above, and this may relate to the quality of their prey. For example, the
277 amphipods and limpets that form the diet of *T. cerberus* generally contained less Σ PUFA, less 22:6 ω 3
278 and had lower 22:6 ω 3:20:5 ω 3 ratios (Sancho et al. 2005; Pond et al. 2008) than those invertebrates
279 known to be eaten by *Symphurus* spp (Tunncliffe et al. 2010; 2013). The relatively high abundances of
280 PUFA in *Symphurus* sp. A liver imply that they have reliable access to PUFA-rich prey.

281 The fatty acid compositions of gonad and muscle tissues of *Symphurus* sp. A could not be
282 statistically differentiated. The combination of fatty acid biomarkers and relatively high PUFA content
283 in these tissues reflected a mixed crustacean and polychaete diet, corroborating the findings of
284 Tunncliffe et al. (2010). Ovaries of *T. cerberus* contained similar relative amounts of PUFA which the
285 authors suggested arose from the preferential deposition of essential fatty acids in these tissues (Pond et
286 al. 2008). It is not unusual that *Symphurus* sp. A muscle and gonadal tissue contained high proportions
287 of PUFA, compared to the liver. This same pattern, also seen in *T. cerberus* and other deep-sea fish
288 (Petursdottir et al. 2008; Pond et al. 2008; Drazen et al. 2009), reflects the dominance of the muscle by
289 polar lipid, a lipid class that is inherently characterized by high proportions of PUFA (Sargent et al.
290 1999). Therefore, the fatty acid profiles in muscle do not reflect diet alone since polar lipid composition
291 can vary according to environmental conditions (e.g., temperature and pressure; Hazel and Williams
292 1990). Mayor et al. (2013) also observed an overlap in the fatty acid compositions of muscle and gonad
293 in abyssal macrourids, which they attributed to the fact that both tissues are dominated by polar lipids.

294 On the Mariana Arc, differences in fatty acid composition among tissues of *Symphurus*
295 *thermophilus* were not obvious and fish size was a confounding factor. The small size and mass of fish
296 D1, as well as its PUFA-poor bacterial signature, suggest that its diet differed from that of other
297 individuals. Tunncliffe et al. (2013) suggest that food webs involving *S. thermophilus* at Daikoku were
298 more strongly based on chemosynthesis than those at other sites on the Mariana Arc (including Nikko).
299 The stable carbon isotopes appear to support this idea since fatty acids in tissues of fish from Nikko
300 were generally more depleted in ^{13}C than those at Daikoku, and had values indicating a photosynthetic,
301 photic zone origin ($\delta^{13}\text{C} \sim -22\text{‰}$; Gebruk et al. 2000). In addition to fish D1, PUFA were generally

302 present in lower levels in *S. thermophilus* tissues (gonad, liver, eyes) relative to fish on the Tonga Arc.
303 Although *Symphurus* spp. from both volcanic arcs have similar diets (Tunncliffe et al. 2010; 2013), it is
304 possible that prey quality (e.g., PUFA content) was lower on the Mariana Arc, especially at Daikoku.
305 The *Fish Traps* site on Volcano-1 was located within the photic zone and may have received regular
306 inputs of marine snow, which would explain the elevated Σ PUFA and essential fatty acid levels in
307 *Symphurus* sp. A. At East Diamante submarine volcano (Mariana Arc), levels of planktonic fatty acids
308 in vent-obligate invertebrates were inversely proportional to sampling depth (Stevens et al. 2015). The
309 sampling sites on both Nikko and Daikoku were below the lower depths at which the tissues of vent
310 obligates contained appreciable photosynthetic signals on East Diamante (i.e., ~350 m; Stevens et al.
311 2015). However, given our small data set and lack of knowledge of the abundance and fatty acid
312 composition of available prey, this is speculative. Furthermore, we did not measure $\delta^{13}\text{C}$ ratios of
313 individual fatty acids in tonguefishes from the Tonga Arc; such data would have allowed direct
314 comparison of the importance of photosynthetic inputs between the two volcanic arcs.

315 The fatty acid composition of *Symphurus* spp. muscle tissue differed from that of the vent-
316 obligate *T. cerberus* in that it contained higher proportions of PUFA, higher relative amounts of 22:6 ω 3
317 and greater 22:6 ω 3:20:5 ω 3 ratios. Maximum relative amounts of PUFA (49%) and 22:6 ω 3 (33%) in
318 *Symphurus* spp. muscle, as well as 22:6 ω 3:20:5 ω 3 ratios (up to 5.5), were similar to those in most other
319 demersal teleosts from temperate waters (Body 1983; Vlieg and Body 1988; Bakes et al. 1995;
320 Guerreiro et al. 2004; Økland et al. 2005), including forage fish and pelagic species known to store large
321 amounts of lipid in muscle (Saito et al. 1997; Pethybridge et al. 2014). Total muscle lipid (mg g⁻¹ wet
322 mass (WM)) in *Symphurus* spp. was similar to levels in deep-sea fish from the Tasman Sea and North
323 Atlantic, although very high lipid levels were reported for some oreosomatid species (Bakes et al. 1995;
324 Økland et al. 2005). In terms of percent lipid content in muscle (% lipid as a function of WM), levels in
325 *Symphurus* spp. were comparable to those of most deep-sea and benthic fish from the Northeast
326 Atlantic, Northeast Pacific and Southwest Pacific, including four non-vent flatfish species from coastal
327 New Zealand waters (Vlieg and Body 1988; Bakes et al. 1995; Økland et al. 2005; Drazen et al. 2009).

328 The fatty acid composition of all analysed tissues of *Symphurus* spp., compared to literature
329 values for both vent-obligate and non-vent teleost species, suggest that in most cases these tonguefishes
330 have a reliable supply of PUFA-rich prey at shallow hydrothermal vents in the western Pacific Ocean.
331 Stable carbon isotope ratios of fatty acids in fish tissues from *S. thermophilus* on the Mariana Arc
332 indicate ingestion of material that originated in the photic zone. Flatfish have specific requirements for

333 essential fatty acids during larval development, in part to ensure proper pigmentation and eye migration
334 during metamorphosis (Hamre et al. 2007), and these needs appear to be met at the shallow
335 hydrothermal vents where *Symphurus* spp. reside. We suggest that inputs from the photic zone may
336 influence the presence and persistence of vent-obligate vertebrates on intra-oceanic volcanic arcs. The
337 variable fatty acid compositions of *Symphurus* spp., particularly among fish on the Mariana Arc where
338 tissue-specific trends could not be discerned, highlight the need to sample a greater number of
339 individuals in future studies of vent-obligate vertebrates.

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