1   **Trophic specialization on unique resources in one of the most celebrated**

2   **examples of sympatric speciation, Barombi Mbo crater lake cichlids**

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24 **ABSTRACT**

25   Divergent ecological selection often results in trophic niche partitioning and is one of the central

26   processes underlying sympatric speciation. However, there are still few studies of niche

27   partitioning in putative examples of sympatric speciation in the wild. Here we conducted the first

28   quantitative study of dietary niche partitioning in one of the most celebrated examples of sympatric

29   speciation, Barombi Mbo cichlids, using stomach contents and stable isotope analyses. We found

30   little evidence for trophic niche partitioning among any Barombi Mbo cichlids, even among the

31   nine species coexisting in sympatry in the littoral zone. Stable isotope analyses supported these

32   conclusions of minimal dietary overlap. However, we did find extraordinary dietary specialization

33   in some species, including spongivory and feeding on terrestrial ants, both unique feeding

34   strategies among freshwater fishes. Stomach contents of the spongivore (*Pungu maclareni*) were

35   20% freshwater sponge, notable considering that only 0.04% of all fishes consume sponges.

36   Overall, we conclude that while there is less trophic niche partitioning than expected among

37   Barombi Mbo cichlids, there is evidence for dietary specialization on rare resources in support of

38   Liem's paradox.

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47 **INTRODUCTION**

48 All adaptive radiations display some level of niche partitioning, a phenomenon in which groups of

49 organisms in a shared environment shift their resource use to reduce niche overlap. While

50 phylogenetic niche conservatism results in ecological similarities among closely related species

51 (McNyset 2009, Losos 2008), adaptive radiations represent the opposite extreme in which rapidly

52 diversifying species occupy a diverse suite of ecological niches (Schulter 2000, Martin & Richards

53 2019, Stroud & Losos 2020). Trophic niche partitioning in particular can allow for the coexistence

54 of similar species by reducing interspecific competition for limited food resources (Ross 1986,

55 Winemiller & Pianka 1990, Correa & Winemiller 2014, Varghese *et al.* 2014). When such niche

56 shifts coincide with traits under disruptive selection and assortative mating, dietary niche

57 partitioning can contribute to ecological speciation in sympatry (Dieckmann and Doebeli 1999,

58 Gavrilets 2004, Gavrilets & Losos 2009). Examples of this phenomenon include experimental

59 evolution studies (Blount *et al.* 2008), divergence of insect host races (Nosil 2009), and ecological

60 speciation in classic adaptive radiations (Grant and Grant 2002; Kocher et al. 2004; Lamichhaney

61 et al. 2015; Gillespie *et al.* 2020). The fine-scale study of dietary niche partitioning and

62 specialization during adaptive radiation can offer further insight into the prevalence and

63 mechanisms of the processes driving ecological speciation.

64 African cichlids are widely regarded as a model system for studying adaptive radiation.

65 The species flocks of Lakes Malawi, Victoria, and Tanganyika in particular contain dietary

66 specialists and closely related species that exhibit varying levels of trophic niche partitioning

67 (Kocher 2004; Martin and Genner 2009; Wagner *et al.* 2009). Despite this, dietary specialization

68 is rarely invoked as the driver of speciation in African cichlids. Habitat partitioning (Albertson

69 2008; Conith *et al.* 2020) and sexual selection (Seehausen 2000; Poelstra *et al.* 2018) are

70   hypothesized to play a much greater role in the observed ecological diversity of these lineages. In

71   fact, there is considerable dietary overlap among many sympatric rock-dwelling Malawi cichlids

72   (Ribbink et al. 1983; Reinthal 1990; Genner *et al.* 1999*a*; Genner *et al.* 1999b; Martin & Genner

73   2009), suggesting that many closely related cichlid species can coexist without strong ecological

74   segregation. It has long been recognized in Lake Malawi that closely related species often show

75   minimal or undetectable ecological differentiation, despite substantial differences in trophic

76   morphology (Liem 1980). This is known as Liem's paradox: trophic specialists act as "jacks-of-

77   all-trades" able to consume both their narrow food source as well as a more generalist diet (Liem

78   1980). A rare exception is observed in the *Alcolapia* species flock of the alkaline Lake Natron

79   (Seegers & Tichy 1999). These three herbivorous species exhibit fine-scale trophic and

80   ecomorphological differences despite limited genomic differentiation, suggesting the importance

81   of trophic niche partitioning in the diversification of this clade (Ford *et al.* 2016).

82   Another African cichlid radiation displaying evidence of trophic niche partitioning is in

83   crater lake Barombi Mbo, Cameroon. This species flock of 11 endemic cichlids is celebrated as a

84   putative example of sympatric speciation in nature and its discovery led to a revival of empirical

85   and theoretical interest in this process (Turelli *et al.* 2001; Schliewen *et al.* 1994; Schliewen and

86   Klee 2004; Coyne & Orr 2004; Bolnick & Fitzpatrick 2007; Richards *et al.* 2019). However, recent

87   work on this system has revealed a complex history of gene flow between Barombi Mbo cichlids

88   and riverine outgroups (Martin *et al.* 2015), with introgression from multiple colonizations

89   potentially contributing to the speciation process (Richards *et al.* 2018).

90   More directly relevant to niche partitioning, there is also evidence for weak disruptive

91   selection within Barombi Mbo cichlids (Martin 2012). Strong disruptive selection is necessary in

92   all theoretical models of sympatric speciation to drive the evolution of reproductive isolation

93   between ecotypes (Dieckmann & Doebeli 1999; Gavrilets 2004; Otto et al. 2008). Compared to

94   the predicted strength of disruptive selection necessary for sympatric speciation (Dieckmann and

95   Doebeli 1999; Bolnick 2011) and empirical estimates of disruptive selection in nature (Kingsolver

96   *et al.* 2001), cichlids of the Barombi Mbo genus *Stomatepia* displayed relatively weak levels of

97   disruptive selection for all trophic morphology traits measured (Martin 2012). While trophic

98   divergence in *Stomatepia* has been previously reported for stable isotope data (Martin 2012),

99   divergence in overall dietary profiles—i.e. stomach contents—has not been assessed since

100   Trewavas *et al.* (1972). Unlike the cichlids of Cameroonian Lake Ejagham—another endemic

101   cichlid lake radiation recognized as an example of sympatric speciation—in which olfactory

102   preferences and sexual selection are hypothesized to drive divergence (Martin 2013; Poelstra *et al.*

103   2018), striking differences among sympatric species in trophic morphology and no sexual

104   dimorphism in ten out of the eleven species in Barombi Mbo suggest that diet could be the primary

105   driver of ecological speciation. Previous qualitative descriptions of diet indicated differences in

106   dietary profiles among some species and identified species that fed heavily on plants and

107   freshwater sponges (Trewavas *et al.* 1972).

108   In this study, we investigated patterns of trophic niche partitioning and specialization

109   among Barombi Mbo cichlids, namely through stomach content and stable isotope analyses. We

110   used stomach content analyses to quantify differences in dietary item proportions, niche width and

111   overlap, and overall dietary composition. We also used stable isotope analyses to investigate

112   relative trophic levels and carbon source differences among species over a longer timeframe than

113   the "snapshot" provided by stomach content data. Investigating dietary and trophic differences and

114   specialization among Barombi Mbo cichlids is the first step in examining whether diet is the

115   primary driver of sympatric speciation in this system.

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117 **METHODS**

118 Study site and sample collection

119 Barombi Mbo is a 1 Mya volcanic crater lake (Cornen *et al.* 1992) in southwestern Cameroon. It

120 is roughly circular in shape with a diameter of 2.5 km and a maximum depth of 110 m, but the

121 oxic zone only reaches to 30 m (Trewavas *et al.* 1972; Cornen *et al.* 1992; Musilova *et al.* 2019).

122 We collected samples in December 2009 through January 2010, and in July through December,

123 2016 from several localities in the lake using a 6 x 2 m seine net with 0.5 cm^2 mesh. *Sarotherodon*

124 *linnellii* and *Konia dikume* were caught by artisanal fishers using gill nets. We collected all 11

125 endemic Barombi Mbo cichlid species. We euthanized captured fish with an overdose of MS-222

126 and immediately took a 5 mg muscle tissue sample from the caudal peduncle for stable isotope

127 analysis. Muscle samples were desiccated individually with magnesium perchlorate in airtight

128 vials following Martin (2012; 2013). Specimens were then individually labeled and fixed in 95-

129 100% ethanol. Field procedures followed approved protocols by the Institutional Animal Care and

130 Use Committees of the University of California, Davis and the University of North Carolina at

131 Chapel Hill.

132

133 Stomach content analyses

134 In total, we selected 241 individuals for stomach content analysis, including at least 8 individuals

135 from each species. Nine out of the lake's 11 endemic cichlid species were analyzed in this study,

136 all except *Sarotherodon caroli* and *Sarotherodon lohbergeri*, which are morphologically and

137 ecologically similar to *Sarotherodon steinbachi*. We removed the entire stomach and intestine

138 from each individual. We then placed stomach contents or a subset of the intestine on a Sedgwick-

139　　Rafter cell containing 1 x 1 mm squares for visualization and quantification under a

140　　stereomicroscope. Dietary proportions were based upon a visual volume estimation method

141　　(Hyslop 1980; Manko 2016). We compressed stomach contents to a uniform thickness (approx.

142　　0.5 mm) and estimated the surface area of each prey item by counting the number of 1 mm^2 squares

143　　covered by the item (Hyslop 1980; Gelwich & McIntyre 2017). Smaller items were assigned

144　　fractions of a square to the nearest 0.1 mm^2. This number was then divided by the total number of

145　　squares covered by all diet items for that individual to calculate individual dietary proportions for

146　　each item. Proportions were rounded to the nearest 0.001 and are reported as percentages.

147　　　　We identified all diet items based on partially digested remnants, including exoskeletal

148　　remains, plant matter, and sponge spicules; unidentified organic matter was classified as detritus

149　　and inorganic matter, such as particles of sand, was classified as silt. All prey items were grouped

150　　into taxa, usually to the level of class or family. Diet categories were comparable to previously

151　　identified prey items of Barombi Mbo cichlids described in Trewavas *et al.* (1972). We used 13

152　　diet categories in total: ants, *Corvospongilla spp.* sponge, Dipteran larvae, Ephemeropteran larvae,

153　　Trichopteran larvae, fish, gastropod shell, nematode, plant tissue, shrimp, detritus, silt, and

154　　unidentified. Ants were identified by distinct head capsules of species within Formicidae, which

155　　likely originated from terrestrial debris that fell into the lake. The sponge category consisted of

156　　two members of the genus *Corvospongilla*: *C. thysi*, endemic to Barombi Mbo, and closely related

157　　*C. bohmii* (Trewavas *et al.* 1972). Both species are found in the lake's shallow waters (up to 3-4

158　　m depth), with *C. thysi* typically covering the outer surfaces of rocks and *C. bohmii* found in

159　　crevices (Trewavas *et al.* 1972). Dipteran larvae included larval forms of the midge families

160　　Chaoboridae and Chironomidae. Ephemeropteran larvae included larval forms of various mayfly

161　　families Baetidae and Caenidae. This category also included larvae of the common burrowing

162   mayfly species *Povilla adusta*, previously identified by Trewavas *et al.* (1972) to be present on

163   both stones and fallen logs in Barombi Mbo. Trichopteran larvae consisted of caddisflies in their

164   larval form, likely from the genus *Triaenodes*, which has many species endemic to West Africa

165   (Andersen & Holzenthal 2002). The fish category was assigned to portions of fish fins and tissue,

166   as well as to whole fry found in individuals' stomachs (not identifiable to the species level at this

167   size). The gastropod shell category consisted of shell remains from various snails, including

168   freshwater limpets from the genus *Ferrissia* (Trewavas *et al.* 1972). The nematode category

169   contained all roundworms, likely including both terrestrial and aquatic species. The plant tissue

170   category was assigned to all plant material found in individuals' stomachs. This included aquatic

171   species such as *Najas pectinate* and *Potamogeton octandrus* previously documented in Barombi

172   Mbo (Trewavas *et al.* 1972) and any terrestrial plant leaves. The shrimp category consisted of

173   *Caridina spp.* and *Macrobranchium spp.*, freshwater shrimp genera found in Barombi Mbo and

174   throughout Cameroon (Trewavas *et al.* 1972). Detritus was used as a catch-all term to describe

175   organic matter that was digested beyond the point of identification. Silt was used as a catch-all to

176   describe inorganic materials, including rocks and sand. Animal remains that could not be clearly

177   identified (e.g. egg-like structures) were grouped into the unidentified category.

178   We estimated dietary niche breadth of each species by calculating Levins' standardized

179   index (Levins 1968) and Pianka's measure of dietary niche overlap (Pianka 1973) using the *spaa*

180   package (Zhang 2016) in R (version 4.0.2). Individuals with empty stomachs were excluded from

181   all calculations ($n = 38$).

182

183   <u>Stable isotope analyses</u>

184   To assess relative trophic positions of Barombi Mbo cichlids, we performed stable isotope analyses

185   for all 11 species (including *S. caroli* and *S. lohbergi*). In limnetic systems, δ13C isotope ratios

186   offer insight into the ultimate carbon source of prey consumed (Post 2002). Higher δ13C values

187   indicate a more littoral carbon source, while lower values indicate a more pelagic source (Post

188   2002). δ15N ratios indicate the relative trophic position of individual consumers (Post 2002). In

189   total, we selected 180 individuals for stable isotope analysis, including at least 6 individuals from

190   each species. Field samples desiccated with magnesium perchlorate in individual vials were

191   subsequently dehydrated at 60° C for at least 24 hours, then 1 mg samples were weighed to the

192   nearest 0.0001 g, packaged into tinfoil capsules, and sent to the UC Davis Stable Isotope Facility.

193   ^{13}C and ^{15}N isotopes were measured on a PDZ Europa ANCA-GSL elemental analyzer interfaced

194   to a PDZ Europa 20–20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, U.K.).

195

196   <u>Statistical analyses</u>

197   Individuals with empty stomachs (n = 38) were excluded from all statistical analyses of stomach

198   contents, leaving a final sample size of n = 203. The sample size for each species is reported in

199   Table 1. We performed all statistical analyses in R version 4.0.2 (R Core Team 2020).

200   To visualize overall dietary similarity among species, we estimated a non-metric

201   multidimensional scaling (NMDS) plot from a Bray-Curtis dissimilarity matrix of dietary

202   proportions for each individual. To test for differences in diet among species, we used analysis of

203   similarities (ANOSIM) with species designated as the grouping variable. To determine which

204   dietary components significantly contributed to the stomach contents of each species, we

205   performed an indicator species analysis (Defrêne & Legendre 1997; Cácaeres & Legendre 2009).

206   This analysis has traditionally been used to identify one or more species characterizing various

207   habitats or sites in ecological studies (Defrêne & Legendre 1997). More recently, it has been used

208   in dietary studies to identify diet items significantly contributing to differences in stomach contents

209   between groups (Hertz *et al.* 2017; Lee *et al.* 2018; Thalmann *et al.* 2020). These visualizations

210   and analyses were performed in R using the *vegan* (Oksanen *et al.* 2019) and *indicspecies* (Cáceres

211   & Legendre 2009) packages.

212   To determine whether individual dietary proportions varied by species, we used

213   generalized linear models (GLMs). All GLMs were fitted using the *stats* package in R (R Core

214   Team 2020). Dietary proportions were first transformed using the arcsine (also known as arcsine

215   square root) transformation typical for proportional data. We fit a separate model for each dietary

216   item after arcsine-transformation of the proportions. The independent variable was species with

217   log-transformed standard length (SL) as a covariate. A normal distribution was used for all models.

218   To test the significance of each model, we performed an ANOVA with Type III sum of squares

219   using the *car* package (Fox & Weisberg 2019) in R. For significant models, we used Tukey's HSD

220   post hoc analysis for pairwise comparisons between species. Post-hoc analyses were conducted

221   using the *stats* package in R.

222   Since volume-based dietary proportions are highly variable depending on prey condition

223   (Buckland *et al.* 2017), we decided to additionally analyze our stomach content data using a

224   frequency of occurrence approach. To determine whether presence/absence of dietary components

225   varied by species, we fit GLMs using a binomial distribution. The proportional response variable

226   was converted into binomial success (proportion > 0) and failure (proportion = 0) and then all

227   models were fit as described above.

228   To determine whether stable isotope content varied by species, we fit GLM models for

229   both δ^{15}N and δ^{13}C. We fit models including all 11 Barombi Mbo cichlids and models including

230   only the three *Stomatepia* species. The response variable for each was $\delta^{15}N$ and $\delta^{13}C$, respectively,

231   and the independent variable was species. A normal distribution was used for all models. To test

232   the significance of each model, we performed an ANOVA with Type III sum of squares. We used

233   Tukey's HSD post hoc analysis for pairwise comparisons between species.

234

235   **RESULTS**

236   <u>Dietary composition and niche breadth</u>

237   We found a majority of Barombi Mbo cichlids consumed detritus, plant tissue, and aquatic insects

238   (Fig. 2). Detritus was the majority (>50%) dietary component in all species except for *S. mongo*

239   (Table 1; Fig. 2). Notably, *Myaka myaka* was the only species with stomach contents consisting

240   of 100% detritus (Table 1), potentially reflecting rapid digestion of its specialized *Chaoborus*

241   larvae diet (Trewavas et al. 1972) into unidentifiable organic matter or capture in 2016 during the

242   summer lekking season when reproductive males may invest all their time in courting females

243   rather than foraging. *Konia eisentrauti* consumed the largest percentage of plant tissue (22.8%)

244   across all species (Table 1; Fig 3). While *Pungu maclareni* and all three *Stomatepia* species

245   consumed shrimp, *Stomatepia mongo* consumed the greatest proportion of shrimp (62.9%) among

246   all species (Table 1; Fig. 1 & 3). This may reflect the rare hunting strategy of this species for

247   nocturnal shrimp prey (also see Lloyd *et al.* 2021 for a nocturnal Malawi cichlid). *S. mongo* were

248   only observed and captured by seine net after twilight hours beginning around 19:00 hours. Most

249   species also had unidentified material in their stomach contents, although this percentage was

250   typically under 15% on average (Table 1). However, *S. steinbachi* contained the highest percentage

251   (38.3%) of unidentified material (Table 1), specifically egg-like structures that could not be

252   identified.

253   Several dietary components were much rarer and found in only one or two cichlid species.

254   *S. linnellii*, the largest species in the radiation, and *Stomatepia mariae* were the only two species

255   to consume fish (Table 1; Fig. 1). *P. maclareni* was the only species to consume gastropod shells

256   and *Corvospongilla* sponge spicules, with the latter component making up about 20% of this

257   species' diet on average (Table 1; Fig. 1). *S. mariae* was the only species to consume ants,

258   comprising about 10% of this species' diet on average (Table 1; Fig. 1). While insect larvae from

259   the orders Ephemeroptera and Diptera were found in the stomach contents of several species, only

260   *Stomatepia pindu* consumed Trichopteran larvae (Table 1; Fig. 2), comprising about 14% of this

261   species' diet on average (Table 1; Fig. 1).

262   *S. linnellii* had the widest niche breadth among all species, whereas *M. myaka* had the

263   smallest (Table 1). Many species displayed considerable dietary niche overlap, with Pianka index

264   values typically ranging from 0.8-1 (Table 2). Notably, *S. mongo* showed the lowest niche overlap

265   with other species, with Pianka index values from 0.2-0.35 (Table 2), possibly reflecting the

266   combination of both temporal and trophic niche divergence in this species.

267

268   <u>Clustering, analysis of similarities, and indicator analyses of overall diet</u>

269   The NMDS ordination (stress = 0.103) displayed little clustering of species by dietary components,

270   with considerable overlap among species (Fig. 3). However, there was a statistically significant

271   difference in overall diet among species (ANOSIM: R = 0.06275, *P* = 0.0238).

272   We identified several dietary items that significantly predicted species identity. Detritus

273   was a significant indicator of *K. dikume, M. myaka,* and *S. linnellii* (indicspecies: Dufrêne-

274   Legendre indicator value = 0.454, *P* = 0.0097). Plant tissue was a significant indicator of *K.*

275   *eisentrauti* (Dufrêne-Legendre Indicator = 0.438, *P* = 0.0199); shrimp was a significant indicator

276   of *S. mongo* (Dufrêne-Legendre Indicator = 0.744, *P* = 0.0003); *Corvospongilla* sponge was a

277   significant indicator of *P. maclareni* (indicspecies: Dufrêne-Legendre indicator value = 0.49, *P* =

278   0.0153); ants were a significant indicator of *S. mariae* (indicspecies: Dufrêne-Legendre indicator

279   value = 0.405, *P* = 0.0448); and Trichopteran larvae were a significant indicator of *S. pindu*

280   (indicspecies: Dufrêne-Legendre indicator value = 0.457, *P* = 0.0271).

281

282   <u>Individual dietary components</u>

283   We found individual diet proportions to vary by species for several items (Fig. 1). Detritus

284   consumption was significantly different among species (ANOVA: $\chi2$ = 36.242, df = 8, P =

285   1.585x10-5). In particular, *M. myaka* consumed about 2 times more detritus than *S. mariae* (Tukey

286   HSD: *P* = 0.044), and *S. linnellii* consumed about 1.5 times more detritus than *S. mariae* (Tukey

287   HSD: *P* = 0.021). Plant tissue consumption varied across species (Fig. 1E; ANOVA: χ^2 = 49.347,

288   df = 8, *P* = 5.455x10^{-8}), with *K. eistentrauti* consuming at least 5 times more plant tissue than *S.*

289   *linnellii*, *S. steinbachi*, *P. maclareni*, *S. mariae*, and *S. pindu* (Tukey HSD: *P* < 0.05). Shrimp

290   consumption also varied among species (Fig. 1A; ANOVA: χ^2 = 116.674, df = 8, *P* < 2x10^{-16}),

291   with *S. mongo* consuming at least 7 times more shrimp than all other species (Tukey HSD: *P* <

292   0.001). Consumption of unidentified diet items varied among species (ANOVA: χ^2 = 55.175, df =

293   8, *P* = 4.082x10^{-9}), with *S. steinbachi* consuming at least 2 times more than all other species (Tukey

294   HSD: *P* < 0.05). *P. maclareni* was the only species to consume *Corvospongilla* sponge (Fig. 1D;

295   ANOVA: χ^2 = 55.461, df = 8, *P* = 3.591x10^{-9}). *Corvospongilla* spicules made up 21.2% of *P.*

296   *maclareni's* diet (Table 1; Fig. 2). Similarly, *S. mariae* was the only species to consume ants (Fig.

297   1B; ANOVA: χ^2 = 51.806, df = 8, *P* = 1.835x10^{-8}). Ants made up 9.8% of *S. mariae's* diet (Table

298   1; Fig. 2). *S. pindu* was the only species to consume Trichopteran larvae (Fig. 1C; ANOVA: χ^2 =

299   58.100, df = 8, P = 1.098x10^{-9}). Trichopteran larvae made up 14.1% of *S. pindu's* diet (Table 1;

300   Fig. 2).

301   Collapsing these proportional data to presence/absence data of individual dietary

302   components (as described above) yielded similar results. Detritus (ANOVA: χ^2 = 24.440, df = 8,

303   P = 0.002), plant tissue (ANOVA: χ^2 = 36.158, df = 8, P = 1.643*10^{-5}), shrimp (ANOVA: χ^2 =

304   19.1124, df = 8, P = 0.014), unidentified items (ANOVA: χ^2 = 45.748, df = 8, P = 2.654*10^{-7});

305   *Corvospongilla* sponge (ANOVA: χ^2 = 55.628, df = 8, P = 3.333*10^{-9}); ants (ANOVA: χ^2 =

306   51.997, df = 8, P = 1.685*10^{-8}); and Trichopteran larvae (ANOVA: χ^2 = 17.6528, df = 8, P =

307   0.024), all varied significantly among species by presence/absence with similar specialists as

308   described above.

309

310   <u>Carbon and nitrogen stable isotopes</u>

311   We found $\delta^{13}C$ values to be significantly different among species when comparing all 11 Barombi

312   Mbo cichlids (ANOVA: χ^2 = 123.36, df = 11, P = 2.2x10^{-16}). *S. lohbergeri* had the highest $\delta^{13}C$

313   value, indicative of predominantly littoral foraging, significantly more than all other species except

314   *P. maclareni*, *S. caroli*, and *S. steinbachi* (Tukey HSD: P < 0.01). Contrastingly, *M. myaka* had

315   the lowest $\delta^{13}C$ value, consistent with its open-water pelagic habitat, significantly lower than all

316   other species except *K. dikume*, *K. eisentrauti*, *S. linnellii*, and *S. pindu* (Tukey HSD: P < 0.05). *S.*

317   *mongo* exhibited significantly higher $\delta^{13}C$ than *S. pindu* when comparing all Barombi Mbo cichlids

318   (Tukey HSD: P = 0.041) and only *Stomatepia* species (Tukey HSD: P = 0.005).

319         $\delta^{15}N$ values were also significantly different among species when comparing all Barombi

320   Mbo species (ANOVA: χ^2 = 67.967, df = 11, P = 2.969x10^{-10}). *K. dikume* had the highest $\delta^{15}N$

321   value of any species, significantly more than *M. myaka*, *S. caroli*, *S. lohbergeri*, and *S. pindu*

322   (Tukey HSD: $P < 0.05$). *S. lohbergeri* had the lowest $\delta^{15}N$ value, significantly lower than all other

323   species except *M. myaka*, *S. caroli*, and *S. steinbachi* (Tukey HSD: $P < 0.05$). There were no

324   significant differences in $\delta^{15}N$ values among *Stomatepia* species. Despite these significant

325   differences in both $\delta^{13}C$ and $\delta^{15}N$ values between species, there was minimal clustering by species

326   when visualizing stable isotope values (Fig. 4).

327

328   **DISCUSSION**

329   We found minimal evidence of overall trophic niche partitioning among Barombi Mbo cichlids.

330   However, we found several dietary specializations on unique resources among all freshwater

331   fishes. In particular, our data suggests that Lake Barombi Mbo harbors a sponge specialist, *P.*

332   *maclareni*, and an ant specialist, *S. mariae*. We also document a nocturnal specialist on shrimp (*S.*

333   *mongo*) and specialists on Trichopteran (caddisfly) larvae (*S. pindu*), and plants (*K. eisentrauti*).

334   Herbivory is common among African cichlids (Ribbink & Lewis 1982; Genner & Turner 2005),

335   and *K. eisentrauti* was previously qualitatively described as a plant specialist (Trewavas *et al.*

336   1972). However, the remaining resource specializations are particularly rare among African

337   cichlids. Nocturnality has only been documented once out of thousands of Malawi cichlids (Lloyd

338   *et al.* 2021) and spongivory is only qualitatively reported from *Coptodon spongotroktis* in Lake

339   Bermin, Cameroon (Stiassny *et al.* 1992). In general, our findings align with the major trophic

340   strategies and specialists outlined by Trewavas *et al*. (1972), providing quantitative data on the

341   differences in dietary component proportions between species.

342

343   Minimal trophic niche partitioning in Barombi Mbo cichlids

344 Our measurements of niche overlap suggest there is not strong evidence for dietary niche

345 partitioning in Barombi Mbo cichlids, with Pianka index values of 84% similarity and higher for

346 most species. Furthermore, while we did find significant differences in $\delta^{13}C$ and $\delta^{15}N$ values

347 between species, there was little evidence of clustering by species. This is not uncommon, as

348 coexistence among ecologically similar species can occur even without fine-scale niche

349 partitioning, particularly within speciose African cichlid communities in the great lakes (Liem

350 1980; Ribbink & Lewis 1981; Martin and Genner 2009).

351 Alternatively, dietary niche partitioning may have been obscured by variability in prey

352 condition. Stomach content analyses are highly dependent on prey condition (Baker *et al.* 2014;

353 Buckland *et al.* 2017). Soft-bodied organisms are likely to digest more quickly than those with

354 chitinous exoskeletons or other similarly tough external features, potentially leading to an

355 overrepresentation of hard-bodied, less digestible organisms (Randall 1967). Furthermore, there

356 may be finer-scale niche partitioning at lower taxonomic prey levels (i.e. genus, species) than what

357 can be detected by microscopic visual analysis of stomach contents. DNA metabarcoding

358 approaches may aid in identifying these finer-scale patterns (Berry *et al.* 2015; Harms-Tuohy *et*

359 *al.* 2016; Jakubavičiūtė *et al.* 2017) and can also account for highly digested prey (Carreon-

360 Martinez *et al.* 2011), though such techniques come with their own suite of challenges and may

361 overestimate the relative importance of certain dietary items (Sakaguchi *et al.* 2017).

362 Another explanation for the minimal trophic niche partitioning we observed could lie in

363 undetected seasonal differences. Our data combined specimens collected during both the wet (July-

364 September) and dry (December-January) seasons in Cameroon across multiple years (2009-10,

365 2016). Prey availability often differs greatly between seasons, especially in tropical systems

366 (Winemiller 1990; Correa & Winemiller 2014). However, dietary profiles of the trophic specialists

367 in Barombi Mbo were generally consistent between seasons and years collected. We conclude that

368 these cichlids may be another example of Liem's paradox, in which trophic specialists act as

369 opportunistic feeders until resources become scarce enough that they use their specialized trophic

370 morphology to feed on unusual resources. All specialists appear to be predominantly feeding on

371 common, shared resources in Barombi Mbo (microinvertebrates, microalgae, detritus) while also

372 supplementing their diet with unique resources during both the wet and dry seasons.

373

374 Spongivory in *P. maclareni*

375 One of our most exciting findings is evidence of sponge specialization in *P. maclareni*. A

376 significant proportion (20%) of this species' diet is freshwater sponges (*Corvospongilla spp.*) and

377 it is the only Barombi Mbo species to consume this diet item. Dietary specialization on sponges is

378 extremely rare among fishes, with only about 0.04% of all fishes (FishBase) consuming sponges

379 (2 out of a total of 13 entries are Cameroon crater lake cichlids). The most notable examples of

380 this feeding strategy are marine spongivores that reside on Caribbean coral reefs (Randall &

381 Hartman 1968; Wulff 1994). Eleven Caribbean reef fishes have been identified as sponge

382 specialists, including angelfish in the genera *Holacanthus* and *Pomacanthus*, trunkfish in genus

383 *Acanthostracion*, and filefish in genus *Cantherhines* (Randall & Hartman 1968). Sponge-eating is

384 even more rare within freshwater systems, with the only two examples found in Cameroon crater

385 lake cichlids of Lakes Barombi Mbo (Trewavas *et al.* 1972) and Bermin (Stiassny *et al.* 1992;

386 Schliewen 2005). Compared to the proportions of sponge found in the stomachs of Carribean reef

387 fish—*Holocanthus spp.* (>96%), *Pomacanthus spp.* (70-75%), *Cantherhines macrocerus* (86.5%)

388 (Randall & Hartman 1968)—the proportion of sponge in *P. maclareni's* stomach is small (20%),

389 but still notable considering it is the only fish in this system to consume freshwater sponges.

390   Furthermore, this proportion is still comparable to some of the other Carribean spongivores,

391   including *Acanthostracion spp.* (11-30%) and *Cantherhines pullus* (30.9%) (Randall & Hartman

392   1968).

393   Sponges are a rare diet item among fish and other vertebrates because they are incredibly

394   hard to consume. Most species in the Phylum Porifera have tough exteriors and skeletons made of

395   spongin, calcium carbonate, and silica—all rigid materials. Spongivorous vertebrates, including

396   the hawksbill turtle (*Eretmochelys imbricata*) (Meylan 1988; Witzel 1983; Eckert *et al* 1999) and

397   several Carribean reef fishes have developed morphological adaptations in the feeding apparatus

398   to aid in biting sponge spicules (Hourigan *et al.* 1989). In particular, *E. imbricata* possesses a

399   narrow, beak-shaped mouth that allows for foraging on sponges in coral reefs (Witzel 1983; Eckert

400   *et al.* 1999) and by scraping against the reef's surface (Blumenthal *et al.* 2009). Several species of

401   Carribean angelfish (*Holocanthus tricolor*, *Pomacanthus paru*, *Pomancanthus arcuatus*) also

402   possess a beak-like mouth and multiple rows of tricuspid teeth used to shear sponge off its substrate

403   (Hourigan *et al.* 1989). *P. maclareni* also appears to have adaptations that may aid in sponge-

404   eating, including short robust oral jaws, large epaxial musculature (particularly when compared to

405   other Barombi Mbo cichlids), and fleshy lips with protruding tricuspid teeth.

406   Spongivory in fishes may have evolved through modification of an algae-eating trophic

407   strategy. Algivores often possess morphological and locomotory adaptations to aid in the biting,

408   shearing, and scraping of algae attached to rocks and other substrates (Hulsey *et al.* 2019;

409   Perevolotsky *et al.* 2020). This specialized feeding apparatus may have been co-opted and

410   modified for a sponge spicule diet, as the functional and locomotory processes of shearing algae

411   and sponge are likely similar. In fact, Caribbean spongivores use their peripheral teeth in a similar

412   manner to tear both algae and sponge (Hourigan *et al.* 1989).

413   Another hurdle to spongivory lies in the difficulty of sponge digestion. Poriferans are

414   typically made of materials (spongin, collagen, calcium carbonate, silica) that are difficult to digest

415   and not nutritionally valuable to vertebrates. Furthermore, many sponges produce noxious

416   secondary metabolites, including alkaloids, terpenoids, brominated compounds, and various acids

417   (Faulkner 1984). Several marine sponges producing these compounds have been proven toxic to

418   fish in lab experiments (Green 1977). To aid in these challenges, spongivorous fish may have

419   morphological and physiological adaptations that allow for sponge digestion. Specifically,

420   adaptations in the gut microbiome may be an essential component to sponge digestion. The gut

421   microbiomes of other vertebrates have allowed for consumption and dietary specialization on rare

422   food items, including vampire finches in the Galapagos (Michel *et al.* 2018; Song *et al.* 2019) and

423   scale-eating pupfish (Heras & Martin 2021). Though the gut microbiome of wild-caught *P.*

424   *maclareni* has been previously sequenced in a large comparative study (Baldo *et al.* 2017; Baldo

425   *et al.* 2019), its functional relevance to spongivory was not assessed. Future research on *P.*

426   *maclareni* and other spongivorous fishes should investigate the core gut microbiome of these

427   species and any potential benefits the microbial community confers for sponge-eating.

428

429   <u>Ant consumption by *S. mariae*</u>

430   Another notable finding of this study was evidence of ant specialization in *S. mariae*. We found

431   that about 10% of this species' diet is ants, and it is the only Barombi Mbo cichlid to consume this

432   item. While *S. mariae* has previously been noted to feed on adult terrestrial insects (Trewavas et

433   al. 1972), this is the first study documenting terrestrial ants as a major component of this species'

434   diet. There are several examples of freshwater fish in tropical systems in which ants have been

435   observed as the majority dietary component, including flagtail *Kuhlia marginate* from Moorea,

436   French Polynesia (Resh *et al.* 1999), and queen danio *Devario regina* from Malaysia (Zakeyuddin

437   *et al.* 2017). Most relevant to this study are several species of Ecuadorian cichlids collected in the

438   Upper Amazon River Basin (Saul 1975). All seven species collected (*Aequidens spp.*, *Crenicichla*

439   *spp.*, *Petenia myseri*) consumed ants, with this food item being the most abundant dietary

440   component in *C. lucius* and *C. macrophthalma* (Saul 1975). Ants were listed as an "occasional"

441   food source for cichlids in this study (Saul 1975), a qualitative description that matches with our

442   quantitative finding of ants making up 10% of *S. mariae's* diet.

443   Terrestrial insects are not uncommon components of fish diets, as they enter lakes and

444   rivers through fallen vegetation. The amount of terrestrial insects introduced into aquatic

445   environments likely depends on vegetative and riparian cover, with insect abundance likely

446   increasing as canopy cover increases (Bojsen 2005; Zakeyuddin *et al.* 2017). Fish skimming the

447   water's surface for food will take up these insects while foraging. This is likely why terrestrial

448   insects are an important food source of known surface foragers (Resh *et al.* 1999; Nakano *et al.*

449   1999). While there have not been many studies on the subject, Sullivan *et al.* (2014) reported that

450   the nutritional quality of terrestrial and aquatic arthropods is similar, particularly with respect to

451   structural chitin—a limiting factor in nutrition as this polymer is prevalent in all arthropods and

452   not easily digested by fish. Various species of terrestrial Cameroonian ants have been found to

453   have high levels of protein (55-75% crude protein), and are rich in iron, zinc, potassium,

454   phosphorus, and various other nutritionally-important minerals (Deblauwe & Janssens 2008). In

455   fact, terrestrial insects, including ants, can subsidize the nutrient pool of small lakes, particularly

456   those which have low primary productivity and are located in heavily forested areas (Mehner *et*

457   *al.* 2005). Overabundance of terrestrial food sources in the diet of aquatic animals can indicate that

458   the lake or stream is lacking in autochthonous nutrients (Saul 1975), as is the case for oligotrophic

459   Barombi Mbo (Kling 1988).

460   It is interesting that *S. mariae* was the only Barombi Mbo cichlid to consume ants, as *S.*

461   *mariae* and *S. pindu* are ecologically similar, hybridize in the lab, and represent the extreme tails

462   of a unimodal distribution for all trophic traits measured (Martin 2012). The answer may lie in

463   potential sensory adaptations that *S. mariae* uses to detect ants and its shoaling mid-water habitat,

464   whereas *S. pindu* is a solitary benthic species that forages within the leaf-litter (Trewavas *et al.*

465   1972). Indeed, the genus *Stomatepia* was named for its highly enlarged canal neuromasts (stomae).

466   Sensory traits of Barombi Mbo cichlids remain understudied, except for recent work showing

467   differences in the visual sensory system and the pattern of photoreceptors among various species,

468   with evidence of differences in opsin gene expression between shallow and deep-water species

469   (Musilova *et al.* 2019). Future studies should investigate additional sensory systems in *S. mariae*

470   and other Barombi Mbo specialists to better understand prey targeting of rare food sources.

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729 **TABLES AND FIGURES**

730 Table 1: Mean proportion of each dietary component and sample sizes by species. BA is Levins'
731 standardized index of niche breadth (Levins 1968).

| Dietary component	Konia dikume	Konia eisentrauti	Myaka myaka	Sarotherodon linnellii	Sarotherodon steinbachi	Pungu maclareni	Stomatepia mongo	Stomatepia mariae	Stomatepia pindu
Ant	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.098	0.000
Corvospongilla Sponge Detritus	0.000 0.889	0.000 0.628	0.000 1.000	0.000 0.865	0.000 0.608	0.212 0.609	0.000 0.143	0.000 0.526	0.000 0.651
Dipteran Larvae	0.111	0.027	0.000	0.000	0.000	0.003	0.000	0.023	0.000
Ephemeropteran Larvae	0.000	0.101	0.000	0.025	0.009	0.088	0.000	0.120	0.000
Fish	0.000	0.000	0.000	0.060	0.000	0.000	0.000	0.074	0.000
Gastropod Shell	0.000	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.000
Nematode	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.015	0.000
Plant Tissue	0.000	0.228	0.000	0.045	0.000	0.016	0.019	0.006	0.000
Shrimp	0.000	0.000	0.000	0.000	0.000	0.008	0.629	0.001	0.091
Silt	0.000	0.012	0.000	0.000	0.000	0.002	0.000	0.000	0.000
Trichopteran Larvae	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.141
Unidentified	0.000	0.005	0.000	0.006	0.383	0.059	0.209	0.137	0.117
n	3	37	6	17	15	63	7	44	11
B$_A$	0.021	0.099	0.000	0.027	0.078	0.112	0.098	0.173	0.095

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734 Table 2: Pianka's measure of niche overlap (Pianka 1973) among Barombi Mbo cichlid species.
735 Values range from 0-1, with 0 being no niche overlap and 1 being complete niche overlap.

|	Konia dikume	Konia eisentrauti	Myaka myaka	Sarotherodon linnellii	Sarotherodon steinbachi	Pungu maclareni	Stomatepia mongo	Stomatepia mariae
Konia eisentrauti	0.926							
Myaka myaka	0.992	0.928						
Sarotherodon linnellii	0.988	0.946	0.996					
Sarotherodon steinbachi	0.840	0.791	0.846	0.847				
Pungu maclareni	0.925	0.894	0.932	0.934	0.838			
Stomatepia mongo	0.209	0.207	0.211	0.213	0.342	0.236		
Stomatepia mariae	0.919	0.894	0.921	0.935	0.910	0.909	0.270	
Stomatepia pindu	0.947	0.887	0.954	0.951	0.899	0.906	0.377	0.920

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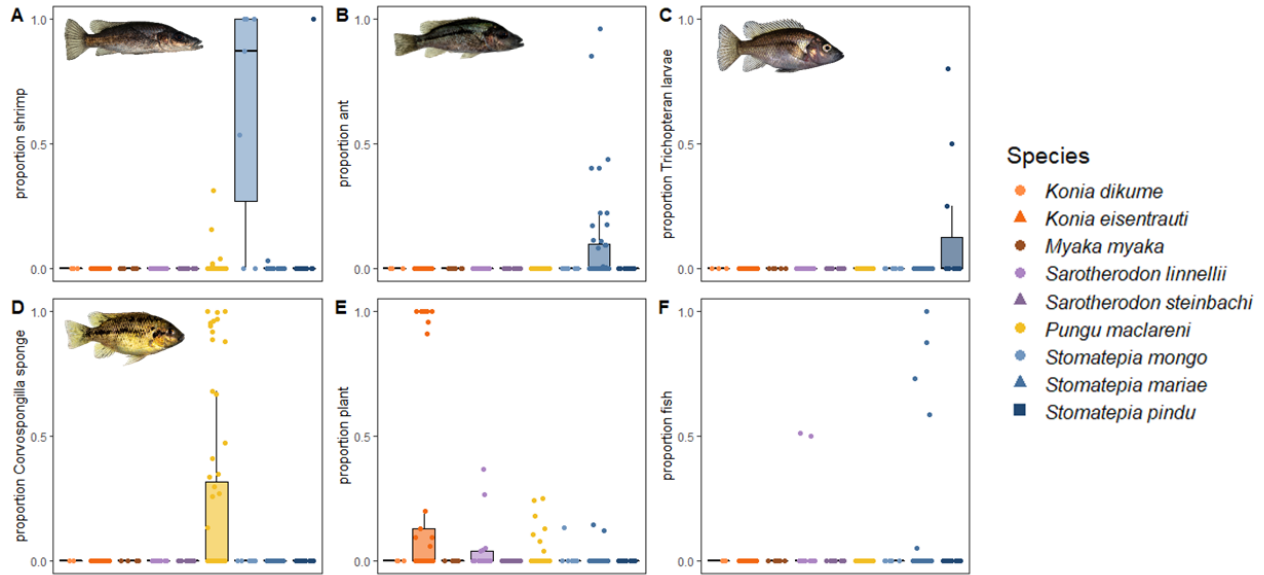
737
738 Figure 1: Box and whisker plots displaying the proportions of A) shrimp B) ants, C)
739 Trichopteran larvae, D) *Corvospongilla* sponge, E) plant tissue, and F) fish found in each
740 species' stomachs. Total sample was 203 individuals collected in 2010 and 2016 from multiple
741 sites around the lake.
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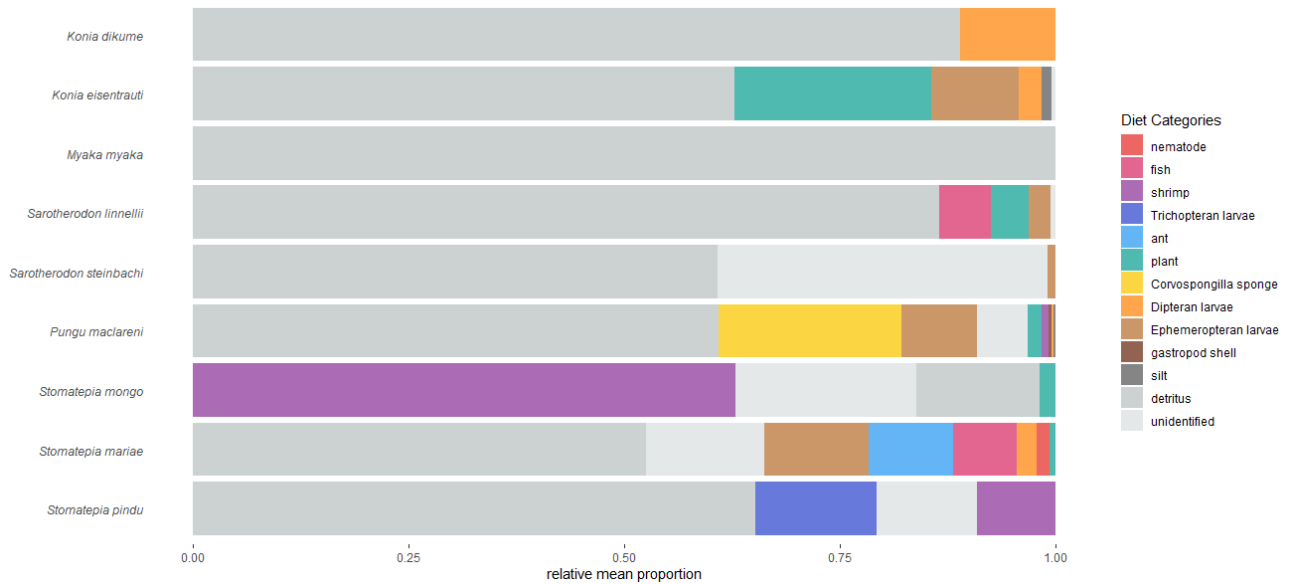
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744 Figure 2: Dietary profiles of Barombi Mbo cichlids by prey item proportion. Each color
745 represents a different dietary component. Bar length is based on the average proportion of each
746 prey item.
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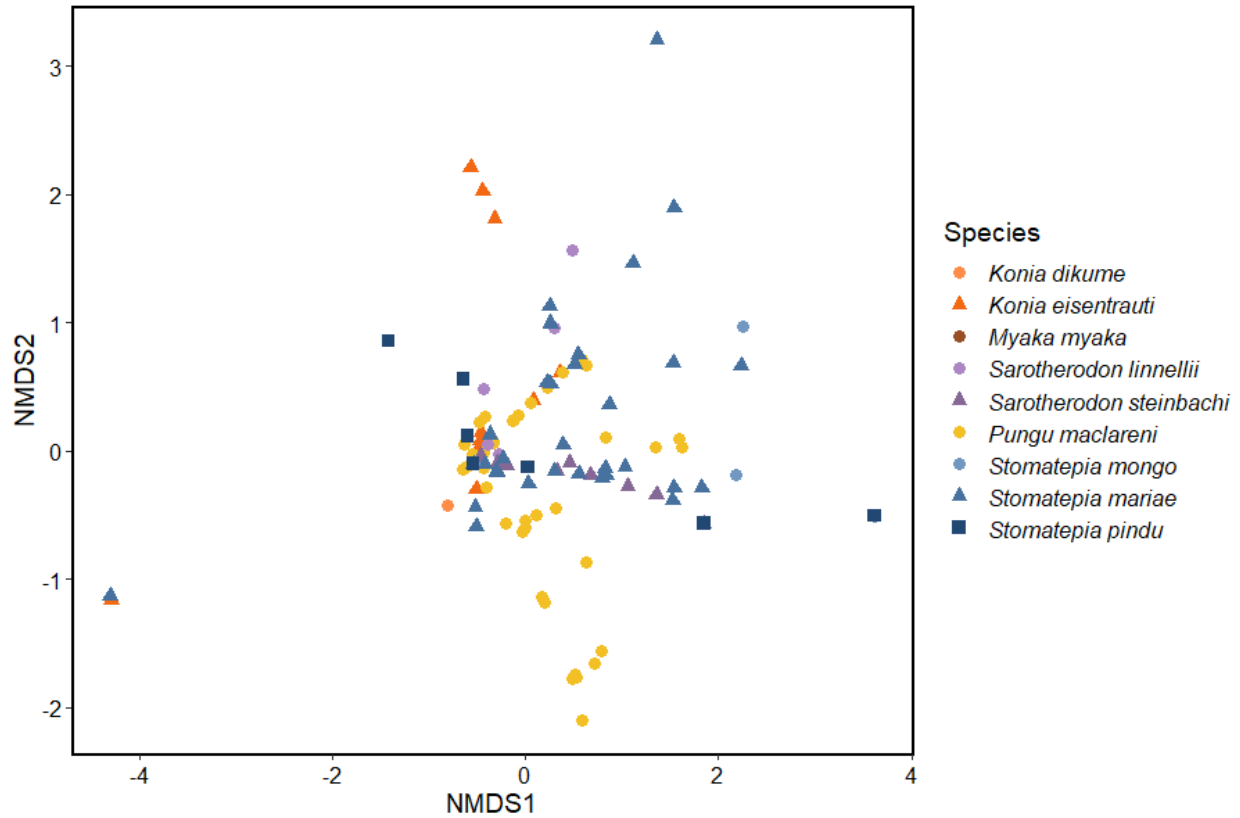
Figure 3: Non-metric multidimensional scaling (NMDS) ordination of dietary item proportions for nine out of the eleven Barombi Mbo cichlids. Ordination is based on Bray-Curtis similarity index (stress = 0.103).
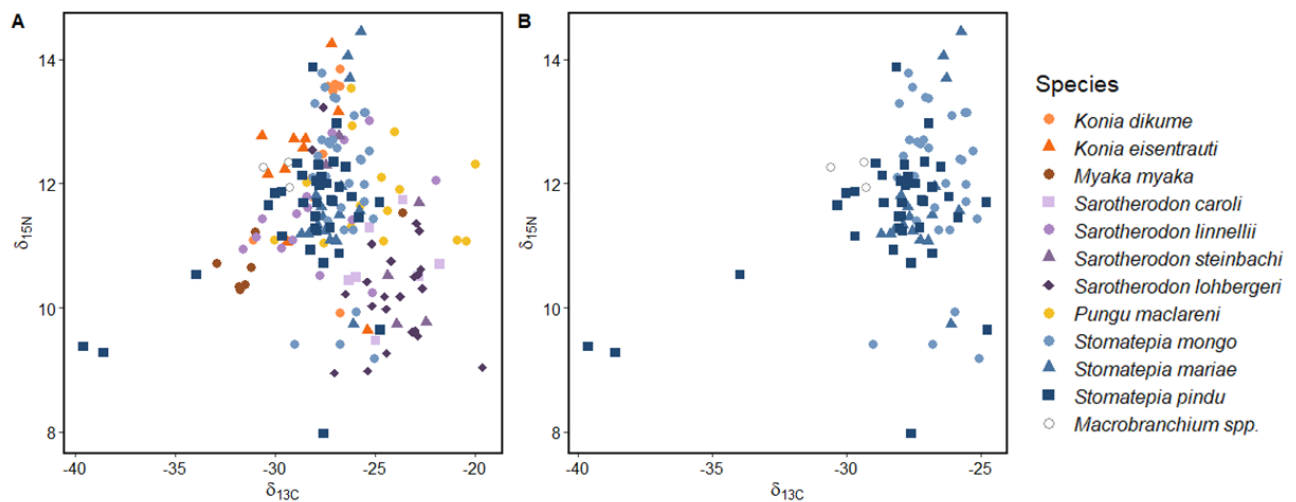
Figure 4: Scatterplots of $\delta^{13}C$ and $\delta^{15}N$ isotopic values for A) all eleven Barombi Mbo cichlids and B) only *Stomatepia* species. $\delta^{13}C$ offers insight into ultimate carbon source (littoral vs pelagic) while $\delta^{15}N$ values describe relative trophic position.