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Trophic Transfer of Lipids and Fatty Acids Across Habitats in Tropical River Food Webs

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Abstract

1. Polyunsaturated fatty acids (PUFA) are essential components of cell membranes and reproductive and sensory organs in vertebrates and are largely acquired through their diets. Accordingly, identification of the dietary sources of PUFA is an important consideration in food web studies.

2. We collected fish, macroinvertebrates (aquatic and terrestrial), and plants (aquatic and terrestrial) from floodplain and river channel habitats in a tropical river catchment in northern Australia, to identify food sources and habitats that provided lipid and PUFA rich food that sustain fish populations.

3. The composition of most FA in fish was similar to that reported from other freshwater tropical environments, with the exception of their higher arachidonic acid content. Fish were found to derive their fatty acids primarily from aquatic sources of food and had similar FA composition to aquatic macroinvertebrates. Aquatic macroinvertebrates fed on aquatic plants (algae) and had higher total lipids and FA contents than plants and fish, providing a more concentrated source of PUFA for fish. Fish obtained most of their FA from their diet except for docosahexaenoic acid (DHA), which they must synthesize due to low DHA in algae and macroinvertebrates.

4. There was no overall difference in basal dietary FA composition between floodplain and river channel habitats. However, macroinvertebrate taxa varied in their lipid and PUFA content.

5. Food quality for fish may therefore vary between habitats as a consequence of differences in macroinvertebrate community composition. Given the high algal production on floodplains compared to the river channels, these habitats are likely to

88   represent the major source of high quality food for fish and other aquatic consumers,

89   and conserving habitats that are rich in lipids and PUFA is important for maintaining

90   healthy fish communities.

91

92

93 **Keywords**

94 Freshwater, Fish, Invertebrate, Algae, Polyunsaturated,

95

1. Introduction

97 In free flowing river systems, fish and other aquatic organisms have the opportunity to move

98 to areas with high quality food that fuels growth and reproduction (Crook *et al.*, 2020;

99 Vasconcelos *et al.*, 2020). Globally, there are relatively few large free flowing river systems

100 remaining (Grill *et al.*, 2019) and understanding the capacity of these systems to provide high

101 quality nutrition for fish to survive and reproduce can provide a target for future restoration

102 and aid in mitigating biodiversity loss (Winemiller, 2004). Lipids and fatty acids (FA) are

103 important biochemical components of aquatic food webs and access to food sources with

104 specific FA profiles helps ensure high quality nutrition is available to support metabolic

105 processes that can influence consumer fitness (Arts, Ackman & Holub, 2001; Swanson,

106 Block & Mousa, 2012). Research to date on the FA of river food webs has largely been

107 focused on temperate regions (Twining *et al.*, 2017; Fujibayashi *et al.*, 2018). Few studies

108 have reported on FA of freshwater food webs in the tropics with the majority of existing

109 tropical freshwater FA studies coming from African lakes (Zenebe, Ahlgren & Boberg, 1998;

110 Kwetegyeka, Mpango & Grahl-Nielsen, 2008; Hecky *et al.*, 2010). It is imperative to

111 understand the production and transfer of lipids and FA through tropical river food webs

112 because biodiversity in tropical rivers exceeds that of temperate rivers but is under increasing

113 anthropogenic pressures (Hawkins, 2001; Jones & Bull, 2020).

114

115 Even though large tropical river systems have high diversity, food web biomass appears to be

116 dominated by a few species connected by short food chains (Winemiller, 2004). While

117 shorter food chains reduce food web complexity, they facilitate efficient dietary energy

118 (including lipids and FA) transfer from primary producers to fish, supporting high fish

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119   biomass (Lewis Jr *et al.*, 2001). Concentration ratios of lipids and FA between trophic levels

120   can indicate how lipids and FA produced by basal food sources (plants, bacteria and fungi)

121   are transferred and retained in aquatic consumers (Arts, Brett & Kainz, 2009). The FA of

122   consumers varies greatly between temperate and tropical environments, suggesting that

123   salinity and temperature play a role in production and transfer of lipids and their FA (Brett,

124   Müller-Navarra & Persson, 2009; Parzanini *et al.*, 2020).

125

126   In free flowing river systems, connectivity facilitates the transfer of dietary energy across

127   habitats, providing access to nutrients that fuel food web productivity and increase biomass

128   (Pettit *et al.*, 2017). In the tropics, fish access highly productive floodplain habitats via lateral

129   connections to creeks and river channels during wet season floods (Jardine *et al.*, 2012;

130   McJannet *et al.*, 2014). These floodplain habitats can serve as aquatic refuges for fish in the

131   dry season when they are disconnected from the river channel (Waltham *et al.*, 2014). High

132   algal biomass in tropical floodplain wetlands supports macroinvertebrate populations that

133   sustain fish biomass through the dry season (Pettit *et al.*, 2011; Venarsky *et al.*, 2020). While

134   floodplain wetlands have been recognized as important in terms of quantity of basal food

135   sources (Pettit *et al.*, 2011; Molinari *et al.*, 2021), few studies exist on the quality of food

136   produced in floodplain wetlands. As such, it is unclear whether access to floodplain habitats

137   is important for obtaining high quality diet, which can be assessed by examining lipids and

138   polyunsaturated fatty acids (PUFA) of primary producers and consumers (Kainz, Arts &

139   Mazumder, 2004; Guo *et al.*, 2017).

140

141   Dietary supply of the essential PUFA alpha-linolenic acid (ALA 18:3n-3) and linoleic acid

142   (LA 18:2n-6) is crucial for aquatic consumers because they cannot be synthesized *de novo* by

143   animals (Bell & Tocher, 2009; Twining *et al.*, 2016). However, fish can convert, but at

144   generally low efficiency, ALA to other omega-3 (n-3) PUFA, such as eicosapentaenoic acid

145   (EPA 20:5n-3) and docosahexaenoic acid (DHA 22:6n-3), and LA to other omega-6 (n-6)

146   PUFA, such as arachidonic acid (ARA 20:4n-6) (Arts *et al.*, 2001). Consumer fitness is

147   influenced by dietary PUFA because they are essential for metabolic functioning, as well as

148   the use of EPA and ARA in growth and reproduction of macroinvertebrates and fishes

149   (Torres-Ruiz, Wehr & Perrone, 2010; Murray *et al.*, 2014), and DHA in fish cognitive and

150   sensory organs such as the brain and eyes (Masuda *et al.*, 1999; Roy *et al.*, 2020). EPA and

151   ARA are also important structural components of cell membranes (Ahlgren, Vrede &

152   Goedkoop, 2009). Freshwater fish may rely on converting ALA to EPA and DHA because

153   freshwater algae often have relatively low EPA and DHA contents (Brett *et al.*, 2009).

154   Therefore, food sources rich in these PUFA are considered high quality foods, and lower

155   trophic level organisms, such as macroinvertebrates, can play a key role in trophically

156   upgrading their food for their own physiological benefit while inadvertently benefiting their

157   consumers (Bec, Martin-Creuzburg & von Elert, 2006; Kainz & Fisk, 2009).

158

159   This study aimed to investigate the sources and transfer of lipids and FA to

160   macroinvertebrates and fishes in freshwater food webs of a free-flowing tropical river system

161   in northern Australia. We also aimed to examine the importance of different macrohabitats

162   for providing fish with a high-quality diet rich in lipids and PUFA. To address these aims, we

163   investigated lipid and FA of food webs in the Mitchell River and its floodplain. Because

164   terrestrial plants are known to contain only traces of EPA, DHA or ARA (Parzanini *et al.*,

165   2020), we hypothesized that a) aquatic food sources would supply these essential dietary

166   nutrients to consumers, and b) floodplain wetlands would provide a higher quality diet to fish

167   than river channel habitats, given the high production of basal food resources in these habitats

168   (Burford *et al.*, 2008; Pettit *et al.*, 2011; Molinari *et al.*, 2021).

169

170

171   **2. Methods**

172   **2.1 Study location**

173   The Mitchell River catchment is situated in the wet-dry tropics of northern Queensland,

174   Australia, and covers an area of approximately 72 000 km^2 (Petheram *et al.*, 2018). The

175   westward flowing main channel of the Mitchell River stretches from the headwaters in the

176   Daintree rainforest in the east to the river mouth in the Gulf of Carpentaria in the west. Major

177   tributaries that flow into the Mitchell River include the Walsh, Lynd, Alice and Palmer rivers

178   (Fig. 1). The geology and river form varies throughout the catchment, with the eastern third

179   comprised of bedrock varying between sedimentary, granitic and volcanic lithology (Batlle-

180   Aguilar *et al.*, 2014). An alluvial delta megafan at lower elevation spreads west from the

181   confluence of the Mitchell and Palmer Rivers, producing a network of braided channels and

182   creeks on the floodplain (Rustomji *et al.*, 2010). Rainfall in the Mitchell catchment is highly

183   seasonal, with only 4 % of annual rainfall (on average) falling across the catchment during

184   the dry season, from May to October (Petheram *et al.*, 2018). The Mitchell River is one of

185   several rivers in northern Australia that has received considerable interest for expansion of

186 agriculture that requires large-scale water resource developments (Commonwealth of

187 Australia, 2015).

188

189 While cattle grazing is currently the most extensive land-use throughout the Mitchell River

190 catchment, there is also a small area of irrigated agriculture in the upper Walsh catchment,

191 supplemented with water from an inter-basin transfer from the eastern-flowing Barron River

192 (Webster *et al.*, 2009). There are several Indigenous Australian communities residing within

193 the catchment (making up ˷23 % of the catchment population) that harvest fish and other

194 animals from wetlands, creeks and river channels as part of their diet (Jackson, Finn &

195 Scheepers, 2014). A mostly unregulated flow regime ensures wet season flows provide lateral

196 connectivity to floodplain habitats such as wetlands and creeks that become disconnected

197 from the river channel in the dry season. Recent research has shown that fish move onto the

198 Mitchell floodplain to feed during the wet season (Jardine *et al.*, 2012), especially larger

199 bodied species (Jardine *et al.*, 2017). Fish retreat to the main channel and wetlands during the

200 prolonged dry season and become more dependent on autochthonous food sources to sustain

201 biomass gained during wet season feeding (Venarsky *et al.*, 2020).

202

203 **2.2 Sample collection**

204 To explore sources of lipids and FA, as well as food quality across different habitats in the

205 Mitchell River catchment, food web samples were collected from river channel, floodplain

206 creek, and floodplain wetland sites. The wetlands were permanent waterbodies that become

207 periodically connected to other parts of the river network during large wet season floods. The

208 catchment is hydrologically connected annually during the wet season and given the lack of

209 instream barriers and floodplain development, fish were presumably able to move freely

210 throughout the system during the wet season. Sampling was limited to the early to mid-dry

211 season and because of large distances between sites and difficulty in accessing remote areas,

212 was conducted over three consecutive years. Most sites were only sampled on one occasion,

213 with the exception of Sandy Creek and Yelko Waterhole, which were sampled in two

214 consecutive years (2018 and 2019; Table 1).

215

216   Fish, macroinvertebrates and plants were collected from 10 sites within the Mitchell River

217   catchment between May and July (late wet/early dry season; Fig.1, Table 1). Only fish were

218   collected from an additional site (White Water wetland). Macroinvertebrates were collected

219   with dip nets and taxa varied based on what was present at each site at the time of sampling.

220   These included aquatic insects, mussels, shrimp and terrestrial insects (grasshoppers).

221   Aquatic plants included periphyton (scraped from sand, mud, wood and/or rock), epiphyton

222   (scraped from macrophytes), phytoplankton (filtered from the water column), aquatic

223   macrophytes (submerged, emergent, floating), and terrestrial plants included grass, leaves and

224   wood.

225

226   Fish were collected by boat or backpack electrofishing during the 2017 and 2018 trips. Cast

227   nets were used to collect fish in 2019. Fish were euthanized, measured and muscle tissue was

228   extracted in the field. The samples from each site that were analyzed for FA were chosen to

229   be representative of the taxa present at the time of sampling. Because we were studying

230   whole communities, sampling was targeted at all habitat types present and effort was

231   allocated to each habitat based on its relative area within the site. All specimens were

232   immediately stored in either charged liquid nitrogen dry shippers or dewars containing liquid

233   nitrogen for transport to Griffith University where they were freeze dried and stored in a -

234   20°C freezer before being sent to the WasserCluster Lunz Research Centre, Austria, for lipid

235   extraction and FA analysis.

236
237   **2.3 Sample preparation and analysis**

238   Lipids and their FA were analyzed as described by Guo *et al*. (2015). Briefly, total lipids

239   from freeze-dried (i.e., all lipids and FA were reported as dry weight; DW) and homogenized

240   samples (terrestrial and aquatic plants: ~10-20 mg, macroinvertebrates: ~5-7 mg, fish tissues:

241 ~15-20 mg) were dissolved in ice-cold chloroform (2 mL) and stored under N2 atmosphere

242 over night at −80°C to improve lipid extraction efficiency. Samples were then further

243 extracted in chloroform-methanol (2:1) and NaCl (0.8 mL; salt wash), vortexed and

244 sonicated, and subsequently analyzed gravimetrically in pre-weighed tin capsules (total lipid

245 content determination). Fatty acids from total lipid extracts and after lipid class separation

246 were derivatized to fatty acid methyl esters (FAME) in a H_2SO_4 methanol solution for 16

247 hours at 50°C. All FAME were stored at -80°C until being separated using gas

248 chromatography (THERMOTM Trace GC) and detected using flame ionization detection

249 (FID). FAME were separated by a Supelco™ SP-2560 column (100 m, 25 mm i.d., 0.2 µm

250 film thickness), identified by comparison to the retention times of known standards (37-

251 component FAME Mix, Supelco 47885-U; Bacterial Acid Methyl Ester Mix, Supelco 47080-

252 U). The FAME concentrations were quantified using calibration curves based on known

253 standard concentrations. All FAME analyses were replicated within the study design and

254 FAME reported as relative (percentage of total FAME) and mass fractions (e.g., mg FAME

255 per g DW) values.

256

257 **2.4 Data analysis**

258 All data analyses were performed using the software Microsoft Excel, Primer 6 (Clarke &

259 Gorley, 2006) and R version 4.0.2 (R Core Team, 2021) using the RStudio IDE (RStudio

260 Team, 2020). FA present as only traces across all samples were removed from the dataset.

261 The FA included in the percentage dataset were BAFA (bacterial fatty acids (sum of: iso-

262 15:0, anteiso-15:0, C15:0, iso-16:0, iso-17:0, C17:0, and C18:1n-7)), 14:0, 16:0, 18:0, 20:0,

263 22:0, 24:0, LA, ALA, C18:1n-9cis, C18:4n-3, ARA, EPA, C22:4n-6, and DHA. A $\log(x + 1)$

264 transformation was applied before performing statistical analyses.

265

266   To assess the lipid and fatty acid among trophic levels, a set of analyses based on Euclidean

267   distances and ratios of FA were used. Terrestrial and aquatic plant and macroinvertebrate

268   species were separated so that the food web groups were: fish (F), aquatic macroinvertebrates

269   (AM), terrestrial macroinvertebrates (TM), aquatic plants (AP), and terrestrial plants (TP).

270   Firstly, the FA in organisms of these river food webs were examined using nMDS to

271   visualize variation among all food web groups and sampling sites. Secondly, contributions of

272   plant and macroinvertebrate food web groups to diet of fish, and plant groups to

273   macroinvertebrates were determined by calculating Euclidean distances among the groups

274   according to the mean values of their FA percentages. Smaller Euclidean distances between a

275   food source and a consumer indicated a greater contribution of that source to the consumer's

276   diet. The Euclidean distances were calculated with the vegan package (Oksanen *et al.*, 2013)

277   in RStudio. Thirdly, FA concentration ratios (AM:AP and F:AM) were calculated for

278   individual FA using the mean content value for each food web group at each site to further

279   elucidate the transfer of lipids and FA through the food webs. Ratios that are close to one for

280   a given FA, indicate a strong connection between the two food web groups for that FA.

281   Finally, the relationships between PUFA and total lipid mass fractions in fish muscle were

282   assessed using linear regressions, to determine whether the long chain PUFA were likely

283   sourced from the diet or from their precursors (ALA and LA).

284

285   To identify the importance of different habitat types in providing high quality diets we tested

286   for differences in fish FA composition among trophic guilds and habitat types (Table 1). This

287   involved a combination of nMDS, PERMANOVA, ANOSIM and SIMPER analyses that

288   were carried out using the Primer software. In the first step, a 3D MDS was used to visualize

289   the variation among trophic guilds according to their FA composition (using Euclidean

290   distances of the $\log(x+1)$ FA percentage data). Subsequently, the presence of significant

8

291   differences among trophic guilds and habitat types was identified using PERMANOVA and

292   specific pairwise differences were identified using ANOSIM. An ANOSIM R value of

293   greater than 0.25 indicates strong significant differences between pairs, while R < 0.25

294   indicates weak differences between pairs. SIMPER was then used to identify which of the FA

295   contributed to any significant differences. Finally, significant differences in lipids and

296   individual FA among habitat types and taxa for fish and macroinvertebrates were identified

297   using ANOVA and Tukey's Honestly Significant Difference tests in RStudio.

298

299 3. Results

300 3.1 Fatty acids across trophic levels

301   Overall, samples were separated into food web groups rather than sampling sites, according

302   to their FA composition, showing that the influence of trophic level on FA composition is

303   greater than the influence of site-specific factors (Fig. 2). Food web groups were strongly

304   significantly different (ANOSIM: R > 0.25, $p < 0.05$ for all pairs of food web groups except

305   TM-TP ($p > 0.05$)). In contrast, overall fatty acid composition was generally not significantly

306   different across habitat groups ($p > 0.05$), aside from floodplain creeks and upstream river

307   channels (ANOSIM: R < 0.25, $p < 0.05$). Among the food web groups, an aquatic and a

308   terrestrial food web pathway were evident in Euclidean distances (Table 2), and these two

309   pathways were separated primarily by differences in EPA and ARA. Terrestrial plants

310   contained little to no EPA, DHA and ARA, and as such grasshoppers also contained little to

311   no EPA, DHA and ARA (Table A1). The EPA and ARA in aquatic plants, though present in

312   low amounts, was likely transferred to aquatic macroinvertebrates and then to fish.

313   Submerged macrophytes and algae (epiphyton and periphyton) were the plant types with the

314   highest ARA content, while submerged macrophytes, algae (epiphyton and periphyton) and

315   phytoplankton contained the highest EPA of all plant types sampled (Table A1). The DHA

316   content was too low for detection in most plant types, except for phytoplankton, which had

317   low levels of DHA compared to aquatic macroinvertebrates and fish. Mean FA and total lipid

318   values of fish species, macroinvertebrate taxa and plant types are given in Table A1.

319

320   Within the fish food web group, FA composition differed among trophic guilds

321   (PERMANOVA: df = 4, Pseudo-F = 7.4, p = 0.001; Fig. 3) and habitat types (df = 3, Pseudo-

322   F = 3.8, p = 0.002), with no significant interaction (df = 11, Pseudo-F = 0.9, p = 0.569).

323   Pairwise comparisons using ANOSIM showed that only river channels were significantly

324   different from other habitat types, and these differences were weak (R < 0.25 for all pairs).

325   Conversely, all pairs of trophic guilds were significantly different with R > 0.25 for seven out

326   of 10 pairs, indicating that selective feeding influenced predator-prey similarities in FA

327   composition. The SIMPER analysis showed the FAs that most strongly contributed to the

328   differences among trophic guilds were EPA, DHA, oleic acid and docosatetraenoic acid. The

329   mean (± SD) ratios of fish DHA:EPA, ARA:EPA, and DHA:ARA, which are important in

330   hormone production, were 8.5 ± 26.9, 9.1 ± 26.6 and 0.9 ± 0.6 respectively. There were no

331   differences in these ratios between habitats or trophic guilds, except for fish DHA:ARA ratios

332   which were significantly higher in herbivore/detritivores than invertivores and

333   invertivore/piscivores (ANOVA: df = 4, F = 8.5, $p < 0.001$).

334

335   **3.2 Trophic transfer of lipids and fatty acids – concentration ratios**

336   The macroinvertebrate to plant FA ratio, used as an indicator of FA bioaccumulation in

337   consumers relative to their plant diet, was high across all FA, except for lignoceric acid and

338   the n-3/n-6 ratio (Table 3). Macroinvertebrates accumulated lipids and the longer-chain

339   PUFA EPA, DHA and ARA from their plant diet, providing a higher quality and more energy

340   rich food for fish than plants (Fig. 4). Conversely, fish did not concentrate lipids and FA from

341   their macroinvertebrate diet, except for lignoceric acid and DHA (Table 3). This indicates

342   that DHA is mostly produced by these tropical fish since the fish cannot obtain required

343   amounts of DHA from the diet because of the low DHA in plants and macroinvertebrates.

344   Fish had similar contents of stearidonic acid (SDA; 18:4n-3), terrestrial FA (20:0 + 22:0 +

345   24:0), and n-3/n-6 ratios to macroinvertebrates. Macroinvertebrates and fish from the

346   Mitchell River system also contained high amounts of ARA (Table 3 & A1).

347
348   **3.3 Fish lipid and fatty acid content**

349   The ALA and LA in the fish muscle tissue was proportional to total lipid content, indicated

350   by significantly positively correlated mass fractions of ALA and LA with total lipids (Fig. 5).

351   Other FA, including myristic acid, stearic acid, palmitic acid, terrestrial FA, saturated FA,

352   MUFA, and bacterial FA were also increased with increasing total lipid content. Conversely,

353   the PUFA EPA, DHA, and ARA of fish muscle were not correlated with total lipid content

354   (Fig. 5).

355
356   **3.4 Taxa and habitat variations in total lipids and PUFA**
357
358   While F-statistics were significant for fish total lipids across habitat types (Table 4), post-hoc

359   Tukey HSD tests were not significantly different among habitat types. Total lipids were

360   highly variable across fish species and macroinvertebrate taxonomic orders (Fig. 6 & 7).

361   There were individual species from most trophic guilds that had mean lipid contents above 80

362   mg g^{-1}, e.g. gulf grunter, archerfish, glassfish and sooty grunter. Equally, some species across

363   guilds had mean lipid contents below 50 mg g^{-1}, e.g. black catfish, salmon catfish, goby,

364   mouth almighty, freshwater longtom and sleepy cod. Despite this variation,

365   invertivore/piscivores had significantly lower total lipids than fish that were invertivores or

366   herbivore/detritivores (ANOVA: df = 4, F = 4.9, p <0.001).

367
368   Because some FA contents were correlated with total lipid content (Fig. 5), percentage data

369   were used to study the FA variation across habitats and species. Fish species differed in their

370   PUFA composition, and the omega-3 and omega-6 combined PUFA composition (Table 4).

371   Only fish EPA, n-3, and n-3:n-6 differed among habitats (Fig. 8, Table 4). Specifically, fish

372   from river channel sites in the mid to lower Mitchell River had significantly higher EPA (7.7

373    $\pm$ 6.6%) than those in other habitats (upper channel: 2.5 $\pm$ 2.2%, floodplain creek: 2.4 $\pm$ 1.8%,

374   floodplain wetland: 2.2 $\pm$ 2.1%), with particularly high EPA in some fish from the Mitchell

375   Mid site (up to 21%). The higher n-3 PUFA and n-3:n-6 ratio in mid-lower river channel sites

376   can be attributed to the proportion of EPA in these groups. Additionally, Tukey HSD post-

377   hoc tests revealed that LA was not significantly different among habitat types.

378

379   Habitats differed in aquatic macroinvertebrate EPA, total n-3 and n-6 PUFA, and n-3:n-6

380   ratios (Table 4). Specifically, Tukey HSD post-hoc tests showed that EPA was lower in

381   macroinvertebrates from floodplain wetlands than other habitat types. However,

382   macroinvertebrate EPA was highly variable across wetland sites, with Twelve Mile Lagoon

383   having the second highest mean EPA content, and Yelko and Leichardt waterholes the lowest

384   EPA. Macroinvertebrates from river channel sites had the highest EPA content but were not

385   significantly different from macroinvertebrates from floodplain creeks. Macroinvertebrates

386   from floodplain wetlands had lower n-3 PUFA and n-3:n-6 ratios, and higher n-6 PUFA than

387   macroinvertebrates from other habitats.

388

389   **4. Discussion**

390   This study demonstrated that aquatic macroinvertebrate selectively retained FA from feeding

391   on aquatic plants and provide a lipid-rich and high-quality food for fish in the Mitchell River

392   system. Our results showed that fish in the Mitchell River were similar in their FA

393   composition to fish in other tropical freshwater regions (Zenebe *et al.*, 1998; Guo *et al.*,

394   2020), but different from temperate, cold-water fish or marine fish (Parzanini *et al.*, 2020),

395   suggesting that water temperature and salinity, in addition to fish taxa, likely play an

396   important role in determining their lipid and FA composition.

397

398   **4.1 Sources and trophic transfer of lipids and fatty acids**

399   Fatty acids are effective tracers of dietary pathways because FA are released, but not

400   generally degraded, from ingested lipid molecules during digestion of prey (Iverson, 2009).

401   They are then taken up and preserved in storage fats (such as in muscle tissue) and can be

402   traced back to food web origins (Iverson, 2009). Multidimensional scaling and Euclidean

403   distance measures revealed two potential diet pathways in the study: Firstly, there was an

404   aquatic pathway through which fish derived most of their FA from consuming aquatic

405   macroinvertebrates, and aquatic macroinvertebrates derived most of their FA from aquatic

406   plants. The second diet pathway was terrestrial, where terrestrial macroinvertebrates

407   (grasshoppers) most likely fed on terrestrial plants, which contributed little to the FA profiles

408   of the aquatic macroinvertebrates and fish.

409

410   Aquatic macroinvertebrates contained long-chain PUFA such as EPA that support fish

411   growth and reproduction, resulting in a similar FA composition between fish and aquatic

412   macroinvertebrates. The similarity of FA between aquatic plants and macroinvertebrates

413   further suggests that macroinvertebrates sequester and retain PUFA-containing algae

414   (phytoplankton, periphyton, epiphyton), which can subsequently get transferred to consumers

415   at higher trophic levels (Guo *et al.*, 2018). Aquatic macroinvertebrates were rich in lipids and

416   PUFA, in particular EPA, compared to plants, providing an energy rich higher quality diet

417   than plants for fish. For some FA, such as ALA and LA, the lower concentrations in fish were

418   proportional to lower total lipid content, and for others it may indicate that fish either use

419   more of the FA than macroinvertebrates for higher functions or do not require them to the

420   same degree as macroinvertebrates. Further, distinct aquatic trophic trajectories influenced

421   the FA composition of fish, suggesting that selective feeding results in higher FA similarity

422   between prey and fish. The higher variation in herbivore/detritivore fish likely reflects the

423   shorter food chain since plants were the most variable food web group, and the FA in fish

424   track their diet. The long chain PUFA ARA in fish was similar to their diet, unlike LA, ALA,

425   and EPA which were low in fish compared to their diet. While DHA was eight times higher

426   in fish than macroinvertebrates, it was low compared to fish from temperate and cold-water

427   regions.

428

429   Lower DHA in tropical fish than temperate and cold-water fish may be due to the lipid

430   composition of the cell membranes, which is thought to be temperature dependent in

431   ectotherms (Hazel, 1984). Even though DHA was low compared to other climates, fish from

432   the Mitchell River had higher DHA than tropical freshwater fish reported elsewhere (Zenebe

433   *et al.*, 1998; Parzanini *et al.*, 2020). It is evident that the fish in this study synthesize their

434   DHA from dietary precursors, such as EPA, because fish have high DHA requirements and

435   only traces of DHA were present in aquatic plants and macroinvertebrates. Fish eyes are

436   concentrated in DHA, including eyes of marine (Stoknes *et al.*, 2004; Hong *et al.*, 2014) and

437   freshwater fish (Ebm *et al.*, 2021), but turbid waters throughout many northern Australia and

438   other tropical rivers mean that fish have to rely more on other senses for predator avoidance,

439   hunting and navigation (Mourente, 2003; Roy *et al.*, 2020). Therefore, both diet and

440   environment are likely to contribute to the lower DHA in northern Australian tropical

441   freshwater fish compared to temperate freshwater or marine fish.

442

443   Comparatively high ARA was also observed in fish from the Mitchell River in this study

444   (Gibson, 1983; Guo *et al.*, 2017). In fact, mean ARA contents in fish were more than twice

445   that Parzanini *et al.,* (2020) reported for all climates in both freshwater and marine

446   ecosystems. This was derived from selective retention (or synthesis) of ARA by aquatic

447   macroinvertebrates, which were on average 95 times more abundant in ARA compared to

448   aquatic plants. Based on the ARA retention in aquatic macroinvertebrates, dietary ARA is

449   efficiently transferred across the plant-animal interface in these tropical freshwater food

450   webs. High ARA contributed to a n-3:n-6 ~ 1 in fish. While increasing ARA accumulation in

451   fish has been observed with increasing water temperatures (Sinclair, O'Dea & Naughton,

452   1983; Norambuena, Rombenso & Turchini, 2016), the dietary requirements for ARA in

453   freshwater fish are not well understood (Bell & Sargent, 2003). Arachidonic acid is important

454   for egg and gonad development in adult fish, and growth, metamorphosis and survival in

455   larval and juvenile fish (Izquierdo, 1996; Asil *et al.*, 2017). The ARA:EPA ratio in fish of this

456   study (9.1 ± 26.6) was higher than reported for African tropical freshwater fish (Suloma &

457   Ogata, 2012). The ARA:EPA ratio is also important because ARA and EPA are precursors

458   for different molecular families of the eicosanoid class of hormones used in immune

459   functions and these hormones compete to inhibit the formation of each other (Sargent *et al.*,

460   1999; Parrish, 2009). Unlike ARA, muscle EPA, ALA and LA in fish from the Mitchell

461   River were similar to tropical freshwater fish reported elsewhere (Parzanini *et al.*, 2020), and

462   lower than subtropical and temperate fish (Guo *et al.*, 2017).

463

464   In contrast to aquatic macroinvertebrates, terrestrial macroinvertebrates (grasshoppers)

465   contained little to no EPA, DHA or ARA required by fish. Similar to other studies, terrestrial

466   plants were found to contain high LA but no EPA, DHA and ARA (Parzanini *et al.*, 2020).

467   While leaves were high in LA, they are difficult to digest due to high cellulose and lignin

468   content (Hixson *et al.*, 2015; Brett *et al.*, 2017). Despite their low dietary quality, terrestrial

469   leaves can provide a substrate for algal growth, which was indicated by the presence of EPA,

470   DHA and ARA in leaf samples collected from the water in this study.

471

472   **4.2 Fatty acids across habitats and taxa**

473   Both macroinvertebrates and fish from river channel sites on the Mitchell River in the mid to

474   lower catchment had higher EPA than those in other habitats. Similarly, Larson *et al*., (2015)

475   found higher PUFA (including EPA) in river channel compared to off-channel fish and

476   macroinvertebrates. Periphyton and epiphyton were high in EPA relative to other plant types

477   and river channel sites provide abundant surfaces for periphyton growth, such as rocks, sand

478   and wood. It is likely that the abundant periphyton in river channel habitats are a primary

479   food source for aquatic macroinvertebrates, even though plants have low EPA compared to

480   macroinvertebrates. High EPA in macroinvertebrates compared to plants is likely due to their

481   preferential retention of algal EPA (Kühmayer *et al.*, 2020), however little is known on their

482   ability to synthesize EPA. Fish may also synthesize some EPA (Murray *et al.*, 2014: as a

483   precursor for DHA), making it difficult to distinguish how much of the EPA in fish came

484   from diet.

485

486   Correlations between PUFA and total lipids have been shown for other wild fish from

487   temperate lakes (Kainz *et al.*, 2017). Because the regulation of FA by fish is not yet fully

488   understood we cannot be certain why these correlations exist for some PUFA but not for

489   others. However, we speculate that the FA which are correlated with lipids within the fish

490   may be derived from the diet. The strong correlations between fish total lipids and LA and

491   ALA but weak correlations of fish total lipids with EPA and DHA suggest that regulation of

492   these FA is different from LA and ALA, and may indicate that EPA and DHA are

493   synthesized by fish from dietary ALA (Bell & Tocher, 2009). It is also possible that long

494   chain PUFA in these tropical fish are regulated by physiological requirements of fish and

495   contents may therefore be independent of dietary supply (Kainz *et al.*, 2017).

496

497   In contrast to EPA, there was no difference in ALA, LA, ARA, DHA or total lipids across

498   macrohabitats for fish and aquatic macroinvertebrates, indicating that river channels,

499   floodplain creeks and wetlands were of equal dietary quality for fish. These results suggested

500   that the overall dietary quality across taxa in each community was not different between

501   macrohabitats. However, the quantity of basal food sources is likely to differ between these

502   habitats. Molinari et al. (2021) found that 94% of the algal production in the Mitchell River

503   catchment occurs on the floodplain, suggesting that floodplain wetlands could support high

504   macroinvertebrate biomass.

505

506   While total macroinvertebrate biomass may determine the quantity of lipids and PUFA

507   available to fish, the abundance of macroinvertebrate taxa present is highly likely to influence

508   dietary quality because total lipids and PUFA were highly variable between

509   macroinvertebrate taxa. Ephemeropterans were rich in total lipids and EPA, while

510   hemipterans and coleopterans were rich in ARA, and decapod shrimp had higher DHA than

511   other macroinvertebrates. The quality of food received by the fish is therefore dependent on

512   the productivity of the system and the macroinvertebrate taxa present (abundance of taxa high

513   in PUFA and taxa low in PUFA) and fish may be advantaged or disadvantaged by the

514   composition of the macroinvertebrate community present at a location (Tao *et al.*, 2020).

515   Further, macroinvertebrate community composition (family-level) is different across

516   microhabitats (sandy or rocky bed, edge, riffle, macrophyte: Thomson *et al.*, 2002), and the

517   overall site abundance of macroinvertebrate taxa (order-level) varies with microhabitat

518   coverage and composition in the Mitchell River (Stewart-Koster *et al.*, 2021). Therefore, both

519   presence/absence and coverage of microhabitat present at a site is likely to influence food

520   quality.

521

522   High densities of macroinvertebrates are associated with macrophytes and floodplain

523   wetlands are known to have high macrophyte biomass (Gregg & Rose, 1985; Thomaz *et al.*,

524   2008). Therefore, wetlands may be able to support higher macroinvertebrate densities than

525   habitats with lower macrophyte coverage, such as flowing river channels. Wetland

526   macrophytes provide habitat structure for macroinvertebrates and a surface for epiphyton

527   growth, and are therefore important for supporting fish biomass through the dry season until

528   the wetlands become reconnected to the river network (Thomaz *et al.*, 2008). The Mitchell

529   River system has relatively unregulated flow and fish are able to use seasonal connectivity to

530   move freely across habitats (Jardine *et al.*, 2012; O'Mara *et al.*, 2021). The pressure to

531   expand agriculture and regulate river flow not only threatens the movement potential for fish,

532   but also threatens food quality through changes in microhabitat leading to shifts in

533   community structure and composition of macroinvertebrates (Bowen, Bovee & Waddle,

534   2003; Leigh *et al.*, 2013).

535

536   **5. Conclusion**

537   This study demonstrated the importance of aquatic algae and macroinvertebrates as a source

538   of lipids and PUFA for fish nutrition in a wet-dry tropical river of northern Australia.

539   Macroinvertebrate communities support fish nutrition in both channel and floodplain habitats,

540   but algal productivity in floodplain habitats is higher than in river channels. Changes to algal

541   productivity and macroinvertebrate community composition may occur as a result of water

542   resource developments and climate change (Bruno *et al.*, 2019), threatening the natural flow

543   of energy through aquatic food webs. A better understanding of lipid regulation and PUFA

544   requirements of tropical freshwater fish will help to inform predictions of the nutritional

545   impacts on fish from climate change and changes to connectivity from water resource

546   developments.

547

560

561   **Data availability statement**

562   Data are available from the authors upon reasonable request.

563
564   **References**

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822 **Figure captions**

823 *Figure 1. Map of Mitchell River catchment and river network. Black points represent sample*

824 *collection locations.*

825 *Figure 2. nMDS of FA composition (%) for all food web components. Symbols represent mean site*

826 *values of food web groups: AM = aquatic macroinvertebrates, AP = aquatic plants, F = fish, TM =*

827 *terrestrial macroinvertebrates, TP = terrestrial plants. 2D stress = 0.18.*

828 *Figure 3. 3D MDS of fish FA composition (%), with samples coloured by trophic guild.*

829 *Figure 4. Mass fractions of A) ALA, B) LA, C) EPA, D) DHA, E) ARA, F) BAFA, for each aquatic*

830 *food web group (AP = aquatic plants, AM = aquatic macroinvertebrates, F = fish).*

831 *Figure 5. Relationships between fish total lipids and the FA A) ALA, B) LA, C) EPA, D) DHA.*

832 *Figure 6. Total lipid mass fractions for each species, coloured by trophic guild.*

833 *Figure 7. Total lipid mass fractions for aquatic macroinvertebrates at the order level.*

834 *Figure 8. (A) EPA, (B) n-3 and (C) n-3:n-6 composition (%) of fish across different habitats. RC =*

835 *river channel, URC = upper river channel, FC = floodplain creek, FW = floodplain wetland.*

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858 **Conflict of interest statement**
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860 The authors declare that they have no known conflicts of interest that could have appeared to
861 influence the work reported in this paper.
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