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Trophic roles of tadpoles in tropical Australian streams

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16 Abbreviated title: Tadpoles in tropical streams

17

19 **Abstract**

20 1. Tadpoles can be abundant consumers in stream ecosystems and may influence the structure

21 and function of streams through their feeding activities and interactions with other organisms.

22 To understand the contribution of tadpoles to stream functioning, and the potential impact of

23 their loss, it is necessary to determine their diets and how they might influence food-web

24 structure.

25

26 2. Using gut content analysis and stable isotope analysis of N and C, we determined the main

27 food sources and trophic positions of tadpoles of five native frog species, invertebrates and fish

28 in upland and lowland Australian Wet Tropics streams.

29

30 3. Omnivory was prevalent amongst the tadpoles and invertebrates. Tadpoles consumed

31 different food according to availability and nutrient quality, but assimilated mainly biofilm and

32 algae. Most tadpoles and invertebrates assimilated the same high-quality foods.

33

34 4. Food webs in upland riffles were simplified by local extinction of tadpoles, and were

35 probably simplified in pools in the cooler months by seasonal decline in tadpole abundance.

36 Food web complexity was increased in some pools by the presence of predatory fish and a

37 greater number of basal sources.

38

39 5. As tadpoles are important seasonal components in stream food webs, their local extinction

40 can greatly alter food web structure and complexity and, possibly, processes such as leaf litter

41 breakdown and sediment accumulation.

42

Introduction

44   To understand functioning of stream systems, especially in the face of environmental

45   change and species loss (Dudgeon *et al.*, 2006), we need community models that incorporate

46   trophic interactions and roles of key taxa from diverse regions of the world (Pearson, Connolly

47   & Boyero, 2015). These interactions and roles may be visualised in food webs that qualitatively

48   or quantitatively illustrate important trophic networks. Trophic models for forested headwater

49   streams, based on gut contents analysis of animals, typically indicate that terrestrial leaf litter

50   provides the energetic and material base of the food web (e.g., Cummins, 1973; Vannote *et al.*,

51   1980; Cheshire, Boyero & Pearson, 2005; Neres-Lima *et al.*, 2017). However, stable isotope

52   analyses of food web components suggest that algae are also important components of these

53   food webs (Bunn, Davies & Mosisch, 1999; Mantel, Salas & Dudgeon, 2004; Dudgeon, Cheung

54   & Mantel, 2010; Rosi-Marshall *et al.*, 2016), highlighting the importance of using a variety of

55   methodological approaches to fully describe food webs.

56   Small streams are characterised by abundant and diverse invertebrates, but tadpoles can

57   also be abundant and are likely to play key roles in food webs and ecosystem function (Allan &

58   Castillo, 2007). Tadpoles feed on algae, sediments, detritus and other animals (Flecker, Feifarek

59   & Taylor, 1999; Ranvestel *et al.*, 2004; Whiles *et al.*, 2006), with some species specialising at a

60   single trophic level, some changing their diets according to resource availability, and others

61   showing ontogenetic dietary shifts (Hocking & Babbitt, 2014). Food quantity and quality for

62   tadpoles may differ among streams (Whiles *et al.*, 2010): thus, while heavily shaded streams are

63   primarily heterotrophic, more open streams tend to be autotrophic, with abundant primary

64   producers such as filamentous algae and diatoms (Anderson & Sedell, 1979). Food availability

65   may also differ among habitats: for example, riffle-associated tadpoles may consume algae and

66   fine detritus, whereas pool-dwelling species in the same stream may consume coarser detritus,

67   which is more readily available in pools (Trenerry, 1988). Epilithic biofilm can also be an

68   important basal food source for tadpoles in both riffles and pools (Trenerry, 1988; Cashins,

69   2009; Whiles *et al.*, 2010; Frauendorf *et al.*, 2013). Stable isotope analysis has shown that some

70   tadpoles are generalist feeders that change their food sources based on availability (Francis,

71   2013; Huckembeck *et al.*, 2014), while others are more specialised (Verburg *et al.*, 2007).

72   Globally, research on the role of tadpoles in stream food webs is limited mainly to the

73   Neotropics (e.g., Verburg *et al.*, 2007; Colon-Gaud *et al.*, 2010; Winemiller *et al.*, 2011;

74   Barnum *et al.*, 2013; Frauendorf *et al.*, 2013). In the Australian Wet Tropics bioregion

75   (hereafter "Wet Tropics"), tadpoles are conspicuous members of the stream fauna through the

76   warmer months (Richards, McDonald & Alford, 1993), and may be major components of food

77   webs. The gut contents of some species have been described (e.g., Trenerry, 1988), but we

78   know little about their dominant food source or what they assimilate. Upland populations of

79   many species, particularly riffle-dwellers, declined or disappeared during the late 1980s and

80   early 1990s as a result of the fungal disease chytridiomycosis (Richards *et al.*, 1993; McDonald

81   & Alford, 1999). These declines may have caused major changes to food webs, as reported for

82   similar declines in the Neotropics (Ranvestel *et al.*, 2004; Colon-Gaud *et al.*, 2008; Connelly *et*

83   *al.*, 2008; Colon-Gaud *et al.*, 2009).

84   We aimed to understand the trophic role of tadpoles in forested stream ecosystems in

85   the Wet Tropics uplands and lowlands, and possible effects of their disappearance. We used

86   stable isotope analysis to determine the basal food sources and the trophic positions of tadpoles

87   and other consumers in riffle and pool communities. We hypothesised that (1) detritus would

88   be the predominant basal food source, in common with other small forest streams; (2) tadpoles

89   would therefore be mainly detritivores; (3) tadpoles would seasonally influence food web

90   structure (in the summer months); and, therefore, (4) loss of tadpoles from riffle sites would

91   greatly simplify food webs compared with adjacent pools.

92

93   **Methods**

94   *Study species, sites, and sample collection*

95   The Wet Tropics is a discrete, biodiverse region in north-eastern Queensland (Pearson

96   *et al.*, 2015; Fig. S1) and has a seasonal climate with a distinct wet season during the summer

97   months, from November to March. Sampling was conducted in Paluma Range National Park in

98   September 2012 and November 2013, and in Tully Gorge National Park in November 2013

99   (Fig. S1), to include representative upland and lowland streams (Pearson *et al.*, 2017). These

100   streams have high diversity of invertebrates (Pearson & Boyero, 2009; Pearson *et al.*, 2017) and

101   moderate diversity (up to seven species) of stream-breeding anurans (Cashins, 2009) (cf. 23

102   species at a Panamanian site; Lips, Reeve & Witters, 2003). Currently, frog species with stream-

103   dwelling tadpoles recorded from Paluma uplands are the pool dwellers *Litoria serrata* and

104   *Mixophyes coggeri*. Historically, two riffles species, *Litoria dayi* and *L. nannotis*, also occurred

105   in this area, but they disappeared in the early 1990s as a result of chytridiomycosis (Richards *et*

106   *al.*, 1993). Species recorded from Tully lowlands are *L. dayi*, *L. nannotis*, *L. rheocola* and *L.*

107   *serrata*, which all remained following the declines in the uplands (C. Hoskin, pers. comm.). The

108   conservation status of these species ranges from least concern (*L. serrata* and *M. coggeri*) to

109   endangered (*L. nannotis*, *L. rheocola* and *L. dayi*) (Hoskin & Hero, 2008).

110   The study streams were perennial and flowed through closed rainforest ("simple

111   notophyll vine forest"; Tracey, 1982). Six riffles and six pools were sampled in each of the

112   study areas (Paluma and Tully). At Paluma, two pools and two riffles were sampled in each of

113   three upland streams (sites P1, P2, and P3), while at Tully three pools and three riffles were

114   sampled in each of two lowland streams (sites T1 and T2) (Table 1). The riffles and pools

115   within a site were 100 – 200 m apart, with a reach length of approximately 10 m each. The

116   streams comprised approximately 15 % riffles, 60 % runs and 25 % pools at Paluma and 30 %

117   riffles, 50 % runs and 20 % pools at Tully. Physicochemical data recorded at each riffle or pool

118   included: pH, conductivity and dissolved oxygen, measured using a Hydrolab Quanta; depth;

119   and percent canopy cover and substratum composition, estimated visually. Current velocity was

120   measured monthly using an Owen's River Hydroprop. Algal cover was estimated visually as

121   percent cover in pools and riffles along a 150-m transect, each month between October 2011

122   and September 2013, as part of seasonal tadpole surveys in streams at Paluma and Tully

123   (Schmidt, 2016).

124   Collection of food web components was undertaken in the late dry season, when

125   streams were at base flow and frog breeding was in progress. In September 2012, we collected

126   invertebrates, tadpoles, fish and basal food sources at the Paluma sites. In November 2013, we

127   collected additional tadpoles at the Paluma sites, and tadpoles and basal sources at the Tully

128   sites. Invertebrates and fish were not collected at Tully for logistic reasons.

129   Dip-net sweeps with a triangular net (mesh size 0.9 x 0.3 mm) were used to collect

130   tadpoles and larger invertebrates from riffles, pools, and any submerged vegetation. The net was

131   bounced along the substratum to force animals into the water column where they could be

132   captured, and loose rocks were dislodged to expose sheltering tadpoles and invertebrates.

133   Sampling commenced at the downstream end of each pool or riffle and continued without a time

134   limit to the other end of the habitat, to ensure that sufficient material was collected for stable

135   isotope analysis (SIA) (minimum 1 mg dry mass per item). Tadpoles were separated by species,

136   weighed and euthanised in a solution of 0.02 % MS-222 buffered with sodium bicarbonate

137   (Braunbeck *et al.*, 2007). Tadpoles not needed for SIA analysis were released. The tadpoles

138   were between Gosner stages 25 and 31 (Gosner, 1960) and were grouped into small, medium

139   and large length classes (Richards, 2002). For *M. coggeri* tadpoles these classes were: < 10 mm,

140   10 to < 22 mm, and ≥ 22 mm, respectively; for other species they were: < 7.5 mm, 7.5 to < 12

141   mm, and ≥ 12 mm, respectively. Invertebrates were rinsed with distilled water. A Smith-Root

142   Model 12B backpack electrofisher was used to catch fish (the eel, *Anguilla reinhardtii*, and the

143   gudgeon, *Mogurnda adspersa*) and large crustaceans (crayfish, *Cherax* sp., Parastacidae; and

144   shrimp, *Macrobrachium* sp., Palaemonidae). Individuals of *A. reinhardtii* were weighed, fin-

145   clipped, then released. Only small individuals of *M. adspersa* were caught, and were euthanised.

146   These mobile species (fish and decapods) are locally resident for most of their lives (Whitehead,

147   1985; Coughlan, 1990; B. Pusey, pers. comm.). All faunal samples were placed on ice and

148   returned to the laboratory.

149   Basal sources collected from each habitat were: leaf litter (whole leaves); coarse and

150   fine particulate organic matter (CPOM and FPOM, > 1mm or < 1mm, respectively); filamentous

151   algae; biofilm; periphyton; and an iron matrix (Blanchette *et al.*, 2014), present only in one

152   pool. The terms "biofilm" and "periphyton" are often used interchangeably to describe the

153   organic layer growing on solid surfaces (Rasmussen, 2010; Ishikawa, Doi & Finlay, 2012;

154   Bunn, Leigh & Jardine, 2013), but we differentiated between them: periphyton comprised

155   mainly algae, recognisable by its green colour, growing on sandy substrata at pool edges, and

156   was removed directly to zip-lock bags; biofilm, which likely comprised a matrix of

157   microorganisms, fine detritus and small amounts of algae (Lock *et al.*, 1984), was collected by

158   scrubbing rocks and washing the material into plastic containers with distilled water. CPOM

159   and FPOM did not accumulate in the riffles, but were collected from pools and washed through

160   sieves of 1-mm and 250-µm mesh to produce samples of about 500 ml each. All material was

161   placed on ice and frozen on return to the laboratory.

162

163   *Sample processing for stable isotope and gut content analyses*

164   In the laboratory, samples were rinsed with distilled water before processing. Tadpoles'

165   guts were removed for gut content analysis. A sample of material from each gut was removed,

166   mixed with a drop of water, placed on a glass slide and identified under a microscope at x 40

167   magnification. The proportion of each food type was estimated as a percentage of the total

168   volume of particles present to the nearest 5 %, following Cheshire *et al.* (2005). For SIA we

169   used whole bodies of small tadpoles (excluding the gut), but only the tail muscle for large

170   individuals (Caut *et al.*, 2013). Tadpoles of different species, and size classes where possible,

171   were kept separate for the analysis, as diet may vary with tadpole stage (Werner & Gilliam,

172   1984).

173   Invertebrates were separated to genus or higher taxon, depending on amount of material

174   available, and into feeding groups (Merritt & Cummins, 1984; Gooderham & Tsyrlin, 2002;

175   Cheshire *et al.*, 2005); taxa from the same feeding group and habitat within an order were

176   combined if necessary to achieve an adequate sample size (Table S5). For most invertebrates,

177   the whole body was analysed because individuals were small. For crustaceans, only the tail

178   muscles were analysed. Fin clips of *A. reinhardtii* were used whole, while *M. adspersa* were

179   analysed with the axial skeleton, scales and guts removed. Isotopic analyses were based on

180   samples of 106 tadpoles (Paluma: 50 *L. serrata*, 14 *Mixophyes coggeri*; Tully: 13 *L. serrata*, 11

181   *L. nannotis*, 12 *L. rheocola*, 6 *L. dayi*), 5365 invertebrates, 3 *A. reinhardtii* and 8 *M. adspersa*.

182   CPOM and FPOM samples were rinsed with distilled water and whole leaves were

183   rinsed, dried and homogenised using a coffee grinder. All samples were oven-dried at 60°C for

184   48 hours and weighed. The invertebrates were weighed to provide estimates of dry mass per

185   taxon. Tadpole wet mass was converted to dry mass using regressions based on samples of 8 to

186   14 individuals per species. For fish, body length measurements were used to estimate dry mass

187   (Pusey, Kennard & Arthington, 2004). All samples were ground to a fine powder using a mortar

188   and pestle, except for some very small samples, which were sent for analysis intact.

189   Analysis for $\delta^{15}N$, $\delta^{13}C$, % N and % C was conducted by the Stable Isotope Laboratory

190   at the University of Hong Kong using a continuous flow stable isotope ratio mass spectrometer

191   (Nu Instruments, Perspective series) connected to an elemental analyser (Eurovector EA3028).

192   Isotope values were normalised with a certified acetanilide reference standard. Vienna Pee Dee

193   Belemnite and atmospheric nitrogen were used as standard references for carbon and nitrogen

194   respectively (Peterson & Fry, 1987).

195

196   *Data analyses*

197   We used ^{13}C as a measure of basal food source and ^{15}N as an indicator of trophic

198   position (Peterson & Fry, 1987). Mean $\delta^{15}N$ and $\delta^{13}C$ ratios of basal sources and consumers

199   were plotted across sites using raw isotopic data (Whiles *et al.*, 2006). For fish with a C:N ratio

200   greater than 3.5, lipid correction was applied to the isotopic carbon values using the equation

201    $\delta^{13}C_{normalised} = \delta^{13}C_{untreated} - 3.32 + 0.99$ (C:N$_{bulk}$), where *normalised* refers to tissues that are lipid-

202   extracted and *untreated* refers to bulk tissues (Post *et al.* (2007). For tadpoles with a C:N ratio

203   greater than 3.5, the correction used the equation $\delta^{13}C_{normalised} = \delta^{13}C_{untreated} - 1.11 + 0.37$

204   (C:N$_{bulk}$), which was derived for tadpoles (Caut *et al.*, 2013). Correction is unnecessary for

205   invertebrates (Kiljunen *et al.*, 2006; Logan *et al.*, 2008).

206   The C:N ratio of basal sources was calculated to compare their nutritional quality (Iwai

207   & Kagaya, 2007), with a lower C:N ratio generally indicating higher quality food (Gulis *et al.*,

208   2004; Iwai, Kagaya & Alford, 2012). Standard deviations for C:N ratios and basal-source

209   contributions were calculated using data per stream site or data from all sites per area if the

210 basal source was found in only one pool or riffle within an area. Standard deviation was not

211 included for the iron matrix C:N ratio because it was found in only one pool. However, for the

212 basal-source contribution of the iron matrix, the mean standard deviation of the other basal

213 sources from the same stream was used to facilitate inclusion in the model (Blanchette *et al.*,

214 2014). Where appropriate, C:N ratios were compared using ANOVA in S-Plus.

215   The relative contribution of basal sources to consumer isotopic signature was modelled

216 with a two-source mixing model using Stable Isotope Analysis in R (SIAR, version 3.2.5). This

217 enables analyses of several basal sources simultaneously to produce the most likely dietary

218 scenarios (Parnell *et al.*, 2010). The model was run using one of two commands, with 500,000

219 iterations, of which the first 50,000 were discarded: we used "siarmcmcdirichletv4" when

220 multiple data points were available for each consumer taxon, and "siarsolomcmcv4" for single

221 data points. Riffle and pool samples of basal sources were combined within sites, but sites were

222 kept separate, and only sources found at a site were included.

223   Basal sources within a site were combined if their carbon isotope signatures differed by

224 less than 1.0 ‰, except that allochthonous and autochthonous sources were always kept

225 separate (Table S1). Other studies have used a threshold of 2.0 ‰ (e.g., Blanchette *et al.*, 2014),

226 but as the signatures of most sources differed by less than this, a smaller threshold was required.

227 Where basal sources within a stream site were combined, the overall average isotopic signatures

228 and standard deviations were used for the mixing models.

229   SIAR requires the input of trophic enrichment factor (TEF) values, which represent the

230 change in ratio of heavy to light isotopes from resource to consumer (Peterson & Fry, 1987), to

231 place the consumers within the source geometry. We used tadpole TEF values of 3.80 ± 0.46 ‰

232 for $\Delta^{15}N$ and 1.19 ± 0.31 ‰ for $\Delta^{13}C$, following Caut et al. (2013). For invertebrates, the $\Delta^{15}N$

233 values used were 0.6 ± 1.7 ‰ for herbivores and 1.2 ± 1.3 ‰ for predators (Bunn *et al.*, 2013).

234 A $\Delta^{15}N$ value of 3.7 ± 2.2 ‰ for predatory fish (Bunn *et al.*, 2013) was used for *M. adspersa*

235 and *A. reinhardtii*, which are known predators (Sloane, 1984; Hortle & Pearson, 1990; Pusey *et*

236 *al.*, 2010). For $\Delta^{13}C$, a TEF value of 0.4 ± 1.3 ‰ was used for invertebrates and fish (Post,

237 2002; Blanchette *et al.*, 2014).

238   Consumers that fell outside the SIAR isotopic mixing space were excluded from

239   analyses and their models were considered to be unresolved (see Blanchette *et al.*, 2014).

240   Sources with a minimum contribution of 20 % were considered "likely" contributors, and

241   sources with a minimum contribution of less than 20 %, and a maximum of greater than 50 %

242   were considered "possible" contributors (Blanchette *et al.*, 2014).

243   The variability of $\delta^{15}N$ in basal sources was standardised by obtaining a baseline

244   relationship between $\delta^{15}N$ and $\delta^{13}C$ for primary consumers, which was used to calculate isotopic

245   trophic position for higher consumers (Vander Zanden & Rasmussen, 1999; Blanchette *et al.*,

246   2014). The baseline equation was obtained from invertebrates usually considered to be primary

247   consumers (grazers, gatherers, filterers or shredders) (Cheshire *et al.*, 2005). Tadpoles are likely

248   to be omnivores (Alford, 1999) and were therefore not included in the equation estimate. The

249   baseline equation for primary consumers was $\delta^{15}N_{base} = 14.224 + (0.344* \delta^{13}C)$, $r^2 = 0.388$, n =

250   33, $P < 0.0001$.

251   Consumer isotopic trophic position (ITP) was calculated using the equation: ITP =

252    $[(\delta^{15}N_{consumer} - \delta^{15}N_{base})/\Delta^{15}N] + 2$, where $\delta^{15}N_{consumer}$ is the isotopic measure of the consumer in

253   question, $\delta^{15}N_{base}$ is calculated from the $\delta^{13}C$ of the consumer using the baseline equation

254   (above), and $\Delta^{15}N$ is the mean trophic fractionation of $\delta^{15}N$ between basal sources and

255   consumers (Winemiller *et al.*, 2011). For $\Delta^{15}N$, a value of 3.8 ‰ was used for tadpoles (Caut *et*

256   *al.*, 2013), 3.7 ‰ for predatory fish (Bunn *et al.*, 2013), and 1.2 ‰ for invertebrates (Bunn *et*

257   *al.*, 2013). ITPs approximating integer values were allocated to that trophic level. Values of 1.9

258   - 2.1 indicated primary consumers and values of 2.9 - 3.1 indicated secondary consumers, while

259   ITPs that were not close to an integer indicated omnivory (Thompson *et al.*, 2007). ITPs were

260   compared using one-way ANOVA followed by Tukey's post-hoc tests in S-Plus, using pooled

261   samples from all sites within each consumer group for tadpoles, herbivorous invertebrates,

262   predatory invertebrates and fish.

263

264   *Food webs*

265   Food webs were constructed for pools and riffles for Paluma sites only, as invertebrates

266   and fish were not collected at Tully. Invertebrate consumers were categorised according to their

267   feeding behaviours (Merritt & Cummins, 1984; Gooderham & Tsyrlin, 2002; Cheshire *et al.*,

268   2005; Whiles *et al.*, 2013). The tadpoles of the two pool species, *L. serrata* and *M. coggeri*,

269   were grouped together in the food webs because they had similar isotopic trophic positions.

270   Trophic positions were assigned using the stable isotope data for consumers, and links were

271   added according to the SIAR analysis. The isotopic trophic positions for invertebrates were

272   variable so were used in combination with information from the literature (e.g., Cheshire *et al.*,

273   2005) to construct food webs.

274   The relative importance of basal resources to the primary consumers was estimated

275   using the mean probability value of the 95 % CI obtained from the SIAR model (basal sources

276   were not combined for this analysis). The values were calculated separately for each primary

277   consumer group and the mean value (per consumer group from all the sites) was calculated as a

278   measure of importance. This was only done for primary consumers because it was not clear

279   whether predators fed on basal sources directly.

280

281   **Results**

282   At the time of sampling, all streams were at base flow. The in-stream habitats differed

283   between Paluma and Tully sites (Table 1), with a larger proportion of boulders, less shade and

284   greater algal growth at Tully. Water quality was similar among streams, except that pH,

285   temperature and nutrient concentrations were lower at Paluma than Tully.

286

287   *Isotopic values of consumers and basal sources across sites*

288   Overlap of $\delta^{13}C$ signatures of basal resources potentially affected the ability to

289   distinguish source contributions to diets (Fig. 1). At Paluma, most of the tadpole and

290   invertebrate groups had $\delta^{13}C$ signatures similar to the basal sources, whereas at Tully, the

291   carbon signatures of tadpoles overlapped only with the autochthonous sources. Consumers were

292   generally $\delta^{15}N$-enriched in comparison to basal sources. Generally, the $\delta^{15}N$ composition ranged

11

293   from about 2 ‰ to 6 ‰ for invertebrates and clustered around 6 ‰ for most tadpoles. The

294   fishes *M. adspersa* and *A. reinhardtii* had the highest δ^{15}N values at Paluma, and *A. reinhardtii*

295   was the most carbon enriched.

296   Tadpole isotopic signatures varied between sampling occasions: they were more δ^{15}N

297   depleted in 2013 than in 2012 at Paluma, and tadpoles from 2013 exhibited more differences in

298   isotopic signatures among species and size classes at both Paluma and Tully (Fig. S2). Isotopic

299   signatures of most invertebrate groups at Paluma were variable: predators and herbivores

300   overlapped in their δ^{15}N and δ^{13}C measures, but gatherers were generally more nitrogen-

301   enriched than grazers (Fig. S3).

302

303   *Source contribution to consumer diets and nutritional quality*

304   Eighteen percent of consumers had unresolved isotopic mixing models due to source

305   overlap and/or omnivory, and 21 % were outside the source mixing space. These included small

306   *L. serrata* tadpoles and *A. reinhardtii* from P2 (Table 2), and a number of invertebrates from all

307   Paluma sites (Table 3). For some consumers, the models were unresolved despite the animals

308   being within the source mixing space – for example, tadpoles at P3 and large *M. coggeri*

309   tadpoles at P1 (Table 2), and several invertebrate taxa, especially at P1 and P3, where more

310   basal sources were available (Table 3).

311   Biofilm was a possible or likely contributor for most tadpoles at P2, but was less

312   evident as a source at P1 and P3 (Table 2). At Tully, the tadpoles were linked to more than one

313   source. Biofilm, algae and/or particulate organic matter were possible or likely sources at T1,

314   whereas filamentous algae, leaves and FPOM were the main sources at T2. Biofilm and

315   filamentous algae had the highest nutritional quality of the basal sources available for

316   consumers (low C:N ratio – Table S2). The iron matrix at P3 was not a possible or likely source

317   contribution to tadpole diets. Particulate organic matter (leaves and FPOM) was a possible

318   contributor for several size groups of several species at Tully, although leaves had the highest

319   C:N ratio, indicating low nutritional quality (Table S2). Moss was a possible food source for

320   tadpoles at Paluma. Periphyton (on sand) was a possible contributor at P1, but had lower

321 nutrient quality than epilithic biofilm at the same site (Table S2). For invertebrates at Paluma,

322 48 % of the SIAR mixing models were unresolved (Table 3), although resolved models

323 indicated biofilm as a possible source.

324   Comparison of the gut content analysis of tadpoles with the results from the stable

325 isotope models suggests that the material assimilated did not correspond closely with that

326 consumed (Tables 2 and 4). High proportions of FPOM or CPOM were found in tadpole guts,

327 with some algae or diatoms, but SIA revealed that tadpoles assimilated mostly biofilm and

328 filamentous algae, depending on availability. The gut content analysis also indicated that some

329 *L. nannotis* tadpoles consumed trichopteran larvae.

330

331 *Isotopic trophic position for consumers*

332   Tadpoles from Paluma in 2012 were all categorised as omnivores (ITP 2.2 – 2.8),

333 whereas in 2013 some were categorised as primary consumers (ITP ~ 2), along with most

334 tadpoles at Tully (Table S3). Both fish species were secondary consumers with ITPs of

335 approximately 3 (Table S4). The ITPs for invertebrates at Paluma were variable both spatially

336 and taxonomically (0.4 - 3.2 for invertebrates generally regarded as herbivores and 1.5 - 4.8 for

337 predators – Table S4). Most of the nominal herbivores were classified as omnivores, but a few

338 were classified as primary consumers (ITP near 2) or secondary consumers (ITP near 3). Most

339 of the crayfish and shrimps were secondary consumers or omnivores. The majority of nominal

340 predators were classified as secondary consumers, but SIA indicated that some fed as omnivores

341 or primary consumers. The ITPs differed among the consumer groups ($F_{4,85} = 7.74$, $P < 0.001$;

342 Fig. 2), with predatory invertebrates having higher ITPs than the herbivorous invertebrates and

343 Tully tadpoles, and fish having higher ITPs than the Tully tadpoles (Tukey's test, $P < 0.05$).

344 There were no differences between Paluma tadpoles and any other group, fish and predatory

345 invertebrates, or herbivorous invertebrates and tadpoles.

346

347 *Food webs*

348   Omnivory was prevalent in the food webs, with primary consumers feeding on various

349   food sources, and secondary consumers feeding across trophic levels (Fig. 3). SIA indicated that

350   grazers, filter-feeders and shredders fed on both autochthonous and allochthonous material,

351   while gatherers and tadpoles consumed a combination of autochthonous and animal material.

352   Predatory invertebrates and fish formed the top trophic level and were linked to all other

353   consumers.

354   The food-web structure at Paluma was more complex (with more links) in pools than in

355   riffles, because tadpoles and fish occurred in pools only (Fig. 3). The biomass of invertebrate

356   shredders and gatherers was higher in pools, that of filterers and grazers was higher in riffles,

357   and predatory invertebrates had similar biomass in both habitats. Food-web structure also

358   differed among the three sites due to varying numbers of basal sources – five at P2 and seven at

359   P1 and P3. Total fish biomass was 1.5 kg at P2 (three *A. reinhardtii* individuals) and 0.011 kg at

360   P3 (six *M. adspersa* individuals). Tadpole biomass (mean dry mass = 10.9 g per sample) was

361   greater than that of the invertebrate consumer groups (mean dry mass = 2.2 g per sample) in the

362   pools of all three sites.

363

364   **Discussion**

365   *Food sources and trophic positions*

366   Generally, the basal sources were the most $\delta^{15}N$ depleted, invertebrates and tadpoles

367   were intermediate, and predatory invertebrates and fish were the most enriched, as expected

368   (e.g., Minagawa & Wada, 1984; Fry, 1988). However, the main sources of assimilated food

369   identified for all consumers (fish, tadpoles and invertebrates) appeared to be biofilm and algae

370   (autochthonous resources), contrary to hypotheses 1 and 2. While this result might be expected

371   for the more open, lowland sites, it was also true of the most shaded, upland sites. We

372   confirmed that biofilm and filamentous algae were the highest quality basal sources (lowest C:N

373   ratios) (Cross *et al.*, 2005), contrasting with allochthonous leaves (highest C:N ratio), and

374   appeared to have been selected whenever available. However, tadpoles and many invertebrates

375   also consumed allochthonous particulate organic matter and were therefore generalist

376   omnivores, feeding across trophic levels (Pimm & Lawton, 1978). Resource use at Paluma

377   overlapped between tadpoles and invertebrates, so competition for food may occur (Morin,

378   Lawler & Johnson, 1988; Pearson *et al.*, 2015), especially if high quality food is in short supply.

379   However, the influence of tadpoles on invertebrate assemblages in Wet Tropics streams is

380   unclear (see below). Omnivory allows tadpoles and invertebrates to maximise resource use

381   (Lancaster *et al.*, 2005) and may be particularly common in tropical streams, in which

382   availability of different food sources may vary with seasonal rainfall (Frauendorf *et al.*, 2013).

383   For example, particulate organic material accumulates during the dry season, but is removed by

384   wet-season floods (Dudgeon, 2000).

385   While gut contents analysis suggests that allochthonous resources are the major food

386   source for consumers in headwater streams (Cummins & Klug, 1979; Cheshire *et al.*, 2005), our

387   results confirm the conclusions of other studies using SIA that autotrophic production may also

388   play an important role (Bunn *et al.*, 1999; Mantel *et al.*, 2004; Dudgeon *et al.*, 2010), although

389   not universally (Neres-Lima *et al.*, 2017). Diets of tadpoles in neotropical streams are similarly

390   generalist, incorporating CPOM, periphyton and non-algal biofilm (Verburg *et al.*, 2007;

391   Barnum *et al.*, 2013).

392   Relative importance of sources can be difficult to assess because of unresolved models,

393   and because we have no estimate of quantity or type of material that is metabolised but not

394   assimilated. Additionally, consumers may selectively assimilate components from a complex

395   mixture of materials such as biofilm, so that the stable isotope signatures of sources and

396   consumers do not completely overlap (Dodds *et al.*, 2014). The isotopic trophic positions of

397   tadpoles at Tully indicated that they were closer to being primary consumers than those at

398   Paluma, probably because the more open canopy of the lowland Tully streams enhanced

399   primary productivity. Invertebrates (analysed for Paluma only), including nominal specialists

400   such as shredders and predators, assimilated a range of sources, but again biofilm and algae

401   were important, as in streams elsewhere (e.g., Jardine *et al.*, 2012; Frauendorf *et al.*, 2013;

402   Hayden, McWilliam-Hughes & Cunjak, 2016). The fish at Paluma were predatory, but their

403   isotopic trophic positions were lower than the predatory invertebrates, possibly indicating short

404   food chains (Jardine, 2016).

405

406   *Food webs and tadpole loss*

407   Different occurrences of consumers and basal sources among our sites and habitats led

408   to spatial variability in food-web structure and complexity (links among consumers and basal

409   sources). For example, the food webs at Paluma were less complex in riffles than in pools

410   because of the absence of tadpoles and fish. The food webs described here are for

411   spring/summer, the period when tadpoles are most abundant. It is probable that declining

412   tadpole abundance in the cooler months also simplifies the food web. During the warmer

413   months, tadpole biomass was greater than that of the invertebrates, suggesting their likely

414   greater importance in the food web and their possible influence on invertebrate assemblages by

415   reducing food availability and/or causing physical disturbance to invertebrates and sediments

416   (Ranvestel *et al.*, 2004; Colon-Gaud *et al.*, 2008; Connelly *et al.*, 2008; Colon-Gaud *et al.*,

417   2009). However, invertebrate assemblages at Paluma do not differ greatly between seasons

418   (Cheshire *et al.*, 2005), suggesting that fluctuations of tadpole abundance have little effect on

419   invertebrate assemblage composition in pools; similar conclusions cannot be drawn for riffles as

420   riffle tadpoles were locally extinct.

421   Tadpoles were a major seasonal influence on food web structure in the summer months,

422   confirming our hypothesis 3. Loss of tadpoles through disease or habitat destruction may thus

423   have a significant impact on community complexity, as suggested by hypothesis 4, and possibly

424   on associated ecosystem processes, especially energy and materials transfer and bioturbation

425   (Ranvestel *et al.*, 2004; Barnum *et al.*, 2013; Frauendorf *et al.*, 2013). Experimental studies are

426   required to further investigate feeding and assimilation by tadpoles, and their interactions with

427   other consumers.

428   Stream food webs are only reliable up to the primary consumer level, after which they

429   consist of a tangled web of omnivores (Thompson *et al.*, 2007). Many studies have used gut

430   content analysis to draw detailed food webs, enabling identification of prey species as well as

431   autochthonous or allochthonous food sources (Mantel *et al.*, 2004; Cheshire *et al.*, 2005;

432   Barnum *et al.*, 2015). Such food webs therefore incorporate consumers at more detailed

433   taxonomic levels, but are unable to show what the consumers assimilate. Our food-web

434   diagrams are simpler representations of the webs that actually exist, given that various nodes

435   include multiple species and given the prevalence of omnivory (Cheshire *et al.*, 2005); however,

436   they benefit from incorporating information on food consumed and assimilated, using SIA and

437   gut content analysis.

438   Our research has demonstrated widespread omnivory among tadpoles and the

439   prevalence of omnivory in Wet Tropics streams. We also highlighted the importance of using

440   both gut content analysis and SIA to construct food webs by indicating the importance of

441   autochthonous food sources to the stream assemblages. In this case, tadpole gut contents did not

442   necessarily indicate what they assimilated, and isotopic trophic position did not always concur

443   with the expected trophic position. Our findings also indicate that absence of tadpoles can

444   greatly alter food webs, which is a major management concern in the light of local extinctions

445   of some stream-dwelling anuran species in the Wet Tropics uplands and globally.

446

455

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660

661 **Table 1**. Characteristics of habitats and sampling sites at Paluma and Tully with ranges where appropriate. The substratum size distribution is presented as proportions (%) of
662 sand/gravel, cobbles and boulders/bedrock, averaged within habitats. Substratum cover, canopy cover, stream depth and temperature measurements were taken at the time of
663 sampling in November 2013. Conductivity, dissolved oxygen and pH readings were measured during the time of sampling in September 2012 (only in pools at Tully). Current
664 velocity and algal cover estimates were obtained from monthly surveys at sites P1, P2, P3, T1 and T2 between October 2011 and September 2013. Nutrient measurements
665 were obtained from water samples collected in February 2012 at Paluma and November 2011 at Tully. No water samples were collected from P1 at Paluma.

666

	PALUMA						TULLY			
	P1		P2		P3		T1		T2	
Stream characteristics	Pool	Riffle	Pool	Riffle	Pool	Riffle	Pool	Riffle	Pool	Riffle
Location	19.00°S, 146.18°E		18.98°S, 146.17°E		18.97°S, 146.17°E		17.77°S, 145.65°E		17.75°S, 145.61°E	
Altitude (m above sea level)	859		795		766		102		237	
Current velocity (ms^{-1})	0.00-0.06	0.05-1.10	0.0-0.09	0.24-1.30	0.00-0.07	0.05-1.30	0.00-0.45	0.25-2.03	0.00-0.41	0.37-1.85
Stream temperature	18.9		20.0		20.0		22.5		22.5	
Conductivity (µScm^{-1})	30	30	31	31	26	26	29	-	35	-
Dissolved oxygen (% saturation)	76.7	78.5	84.2	85.9	80.5	81.1	96.0	-	88.7	-
pH	6.35	6.45	6.70	6.70	6.35	6.40	8.40	-	8.10	-
Filterable Reactive Phosphorus (µgL^{-1})	-		4		4		3		3	
Nitrate and nitrite N (µgL^{-1})	-		46		17		136		226	
Stream depth (cm)	43	10	55	13	62	9	37	29	50	22
Substratum composition – Sand/gravel: cobbles: boulders/bedrock (%)	72:15:13	72:15:13	40:15:45	52:18:30	87:5:8	82:18:0	16:22:62	10:28:62	16:34:50	10:20:70
Canopy cover (%)	50-75	50-75	50-75	50-75	75-100	25-50	25-50	0-25	0-25	25-50
Algal cover (%)	0-25	0-25	0-25	0-75	0-25	0-25	0-100	0-25	0-100	0-25

667

677

| Species | Size class | PALUMA | | | | | | TULLY | |
| | | P1 | | P2 | | P3 | | T1 | T2 |
		2012	2013	2012	2013	2012	2013	2013	2013
L. serrata	Large	-	-	B	-	-	-	B, A, LF	-
	Medium	M	CF	**B**	M, LCF	nr	A	-	-
	Small	-	-	nr[1]	**B**	-	-	-	-
	Small/ medium	-	-	-	-	nr	**LCF**	-	**A**, LF
M. coggeri	Large	nr	-	B	-	nr	-	-	-
	Medium	BP	-	M*	-		-	-	-
L. nannotis	Large	-	-	-	-	-	-	B, A, LF	A, LF
	Small/ medium	-	-	-	-	-	-	B, LF	-
L. rheocola	Large	-	-	-	-	-	-	**B**, A	-
	Medium	-	-	-	-	-	-	B, A, LF	-
	Small	-	-	-	-	-	-	B, A**	
L. dayi	Large	-	-	-	-	-	-	B, LF	-
	Small/ medium	-	-	-	-	-	-	**B**, LF	-

678

679   * Sample from sites P2 and P3

680   ** Sample from sites T1 and T2

681 **Table 3**. Stable isotope mixing model results (using SIAR) for invertebrates and fishes at Paluma stream
682 sites P1, P2 and P3 in 2012. Basal source abbreviations: A = filamentous algae, B = biofilm, C = coarse
683 particulate organic matter, F = fine particulate organic matter, Fe = iron matrix, L = leaves, M = moss,
684 and P = periphyton. Sources not separated by commas were combined for analysis. Boldface source =
685 likely source contribution (minimum contribution $\geq$ 20 %), regular type = possible contribution
686 (minimum contribution > 0 % and maximum contribution $\geq$ 50 %), nr = unresolved (equal source
687 contribution or isotopic source overlap), and nr[1] = consumer outside the basal source mixing space (not
688 analysed using SIAR). Several taxa were combined within feeding groups to produce sufficient material
689 for analysis, as indicated.

690

Major taxon	Family or species	Feeding group	P1	P2	P3
Diptera	Simuliidae	Filterer	BP	-	nr
	Mixed	Filterer	-	B, LCF	-
Several gatherer/filterers	Mixed	Gatherer/filterer	nr[1]	B	-
Parastacidae, large		Gatherer	nr[1]	nr[1]	nr[1]
Parastacidae, medium		Gatherer	nr	nr[1]	nr[1]
Parastacidae, small		Gatherer/predator	nr[1]	nr[1]	nr[1]
Palaemonidae		Grazer	-	-	**B**
Coleoptera	Psephenidae	Grazer	A	nr	-
Ephemeroptera	Leptophlebiidae	Grazer/shredder/gatherer	nr	B, M, LCF	nr
	Mixed	Grazer	nr	M	A
Plecoptera	Gripopterygidae	Predator/grazer	-	M	A
Trichoptera	Mixed	Grazer/gatherer/filterer	BP	-	-
	Philopotamidae	Grazer/gatherer/filterer	-	B	-
	Mixed	Shredder	CF, BP	B, LCF	B
Lepidoptera		Grazer/shredder	-	B, M, LCF	-
Several predators	Mixed	Predator	M	B	-
Coleoptera	Dytiscidae	Predator	-	B	nr
	Mixed	Predator	BP		-
Ephemeroptera	Ameletopsidae	Predator	nr	B	-
Plecoptera	Mixed	Predator/grazer	A	-	-
Hemiptera	Gelastocoridae	Predator	-	nr[1]	-
	Mixed	Predator	nr[1]	B	B
Megaloptera	Corydalidae	Predator	nr[1]	-	B
Arachnida	Pisauridae	Predator	nr[1]	-	nr[1]
Zygoptera	Synlestidae	Predator	nr[1]	B	-
	Mixed	Predator	nr[1]	B	nr
Epiproctophora	Gomphidae	Predator	-	B	-
	Synthemistidae	Predator	-	B	-
	Telephlebiidae	Predator	-	B	-
	Mixed	Predator	nr	-	nr
Trichoptera	Mixed	Predator	nr	B	-
Pisces	*Mogurnda adspersa*	Predator	-	-	B
	Anguilla reinhardtii	Predator	-	nr[1]	-

691

692 **Table 4.** Gut contents of tadpoles at Paluma, collected in 2012 and 2013, and Tully, collected in 2013.
693 The proportions of the various sources are presented as a percentage of overall gut content. Algae and
694 diatoms were not differentiated. Tadpole size classes as in Table 1. FPOM = fine particulate organic
695 matter, CPOM = coarse particulate organic matter.
696
697

Species	Size	Area	FPOM (%)	CPOM (%)	FPOM/CPOM (%)	Algae/diatoms (%)	Invertebrates (%)
L. serrata	Large	Paluma			100		
		Tully			60	40	
	Medium	Paluma	65	25		10	
		Tully			75	25	
	Small	Paluma			90	10	
		Tully			75	25	
M. coggeri	Large	Paluma		75	20	5	
	Medium			75	25		
L. nannotis	Large	Tully	58	20		20	2
	Small/medium	Tully	85	10		5	
L. rheocola	Large	Tully	50	30		20	
	Medium	Tully	60	30		10	
	Small	Tully	60	20		20	
L. dayi	Large	Tully	80	10		10	
	Small/medium	Tully	80	10		10	

698　**Figure legends**

699

700　Figure 1. Mean (± s.d.) δ^{15}N and δ^{13}C ratios of food-web components in (a) Paluma and (b)

701　Tully streams. Abbreviations: A = filamentous algae, B = biofilm, C = CPOM, F = FPOM, Fe =

702　iron matrix, L = leaves, M = moss, P = periphyton (basal sources), and E = *Anguilla reinhardtii*

703　(eel), Ma = *Mogurnda adspersa* (fish). Each consumer point represents a taxon of a specific

704　feeding group for invertebrates, a species of fish, or a size class of a species for tadpoles.

705

706　Figure 2. Isotopic trophic positions (calculated as per Winemiller *et al.*, 2011) of tadpoles (all

707　species) at Tully (Tadpoles Tul) and Paluma (Tadpoles Pal); invertebrates at Paluma –

708　herbivores including all non-predators (Herb inverts) and predators (Pred inverts); and fish

709　(both species combined) at Paluma. Groups that were not significantly different are indicated by

710　vertical lines (Tukey's test with $\alpha = 0.05$). The left and right boundaries of the boxes represent

711　the 25th and 75th percentiles respectively, the central bar represents the median, the whiskers

712　represent the 10th and 90th percentiles, and the dots indicate outliers.

713

714　Figure 3. Food webs linking major community components for riffles and pools at three sites

715　(P1, P2 and P3) at Paluma in 2012-13. The boldface letters at the base of each figure represent

716　basal sources that were present at each site and analysed for δ^{15}N and δ^{13}C content. Weighted

717　links were drawn between primary consumers and basal sources. The line thickness of links

718　between primary consumers and basal sources is based on the relative importance of sources to

719　consumers, using four categories: 1 ($\leq$ 10 %), 2 (11-25 %), 3 (26-50 %) and 4 (> 50 %).

720　Components (boxes) and links that were absent are shown in grey. Boxes are scaled in

721　proportion to biomass of each component in the streams. Basal sources: A = filamentous algae,

722　B = biofilm, C = CPOM, F = FPOM, Fe = iron matrix, L = leaf litter, M = moss and P =

723　periphyton. Invertebrate consumer groups: FF = filter feeders, Ga = gatherers, Gr = grazers, Sh

724　= shredders, and Pr = predators. Other consumers: Fish = *Anguilla* + *Mogurnda*, and T =

725　tadpoles.

726

Supplementary information

727

728

729   Figure S1. Location of study streams in the Queensland Wet Tropics.

730

731   Figure S2. Mean (± s.d.) $\delta^{15}N$ and $\delta^{13}C$ ratios of tadpoles at (a) Paluma in 2012 and 2013, and

732   (b) Tully in 2013.

733

734   Figure S3. Mean (± s.d.) $\delta^{15}N$ and $\delta^{13}C$ ratios of invertebrate herbivores and predators at Paluma

735   in 2012.

736

737   Table S1. Basal sources present in the streams at Paluma and Tully.

738

739   Table S2. The C:N ratio ± SD of basal sources in the streams at Paluma and Tully.

740

741   Table S3. Isotopic trophic positions (ITPs) for tadpoles at Paluma in 2012 and 2013, and Tully
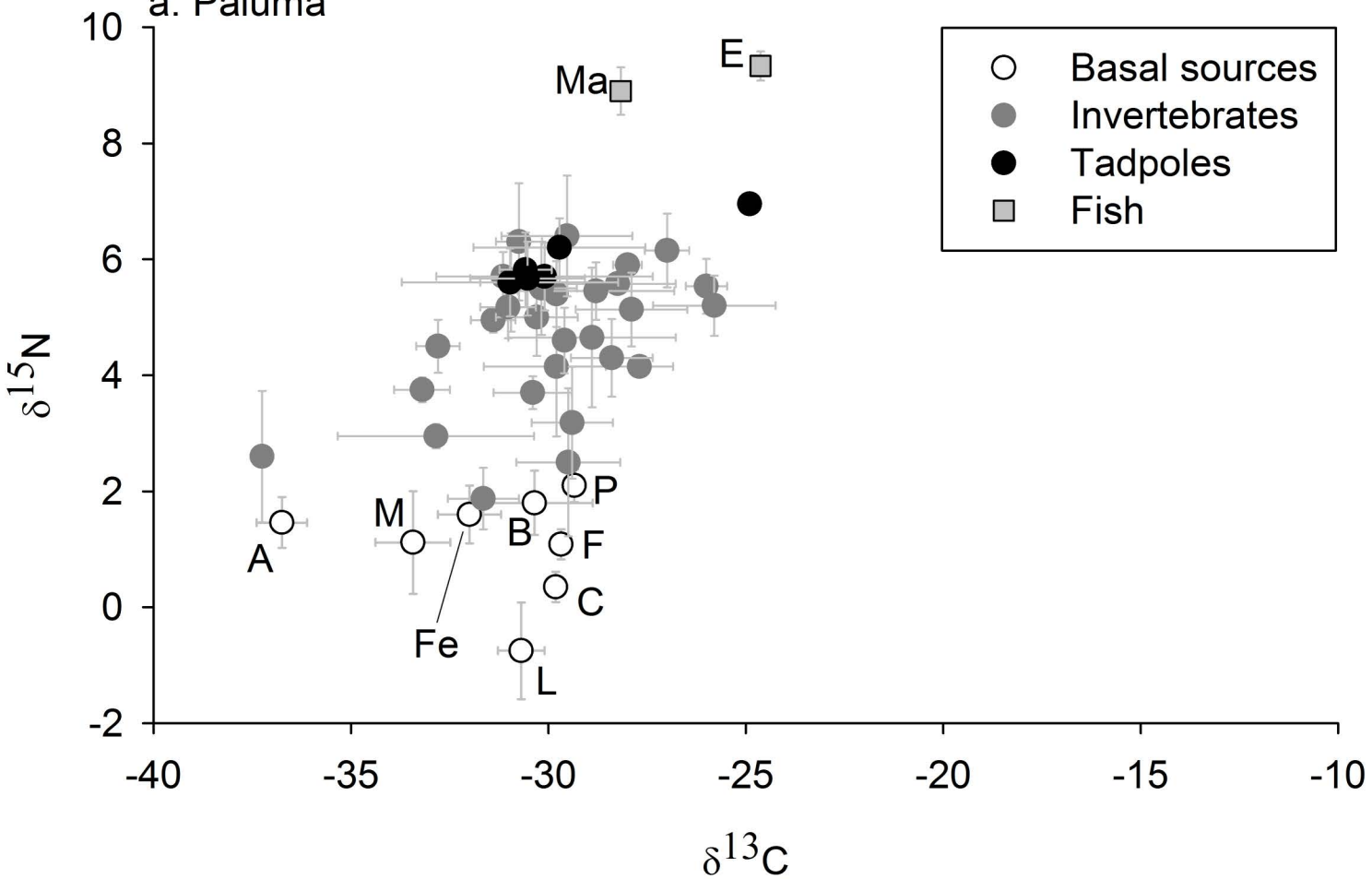
742   in 2013.

743

744   Table S4. Isotopic trophic positions (ITPs) for invertebrates and fishes at Paluma in 2012.
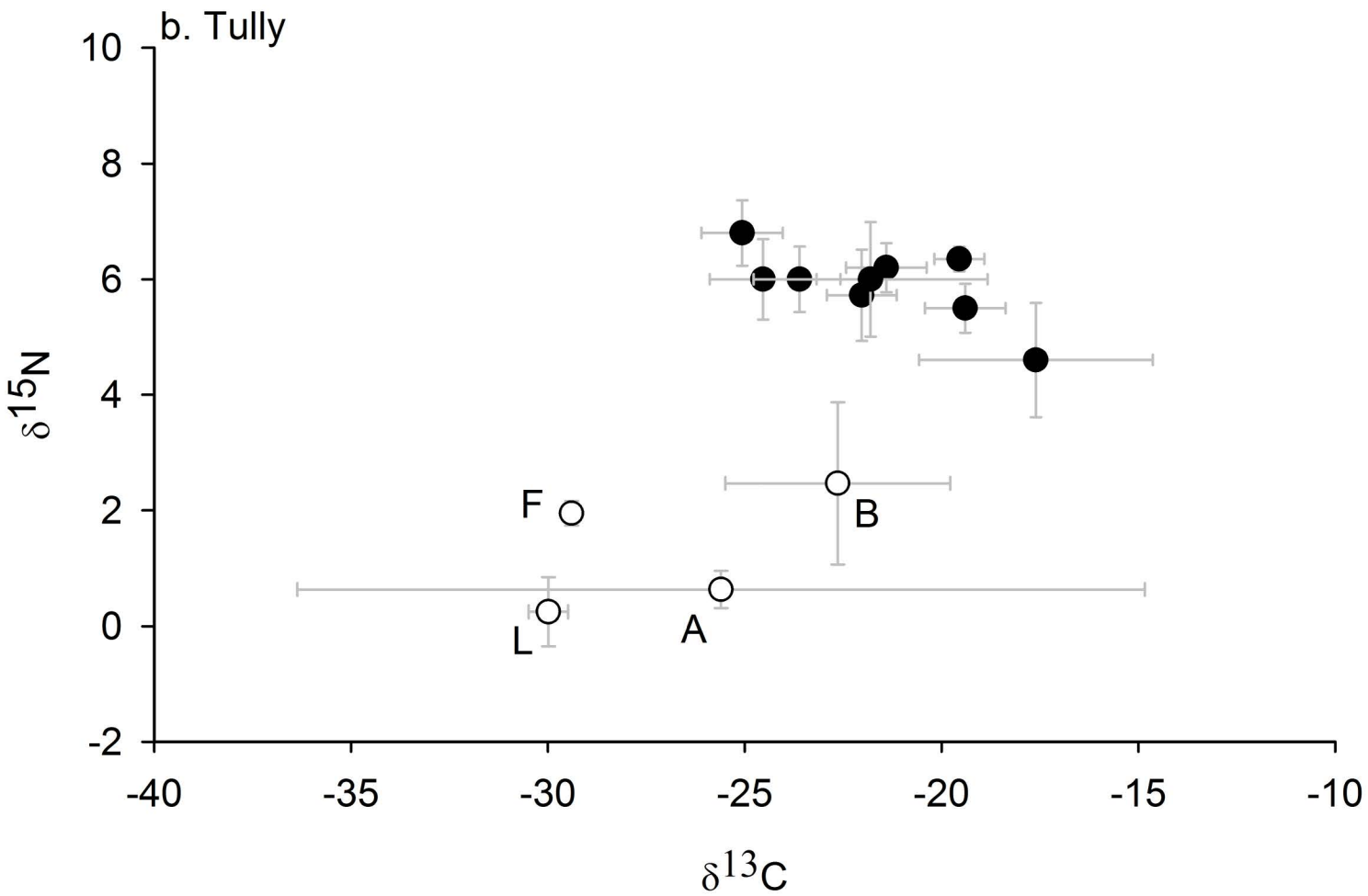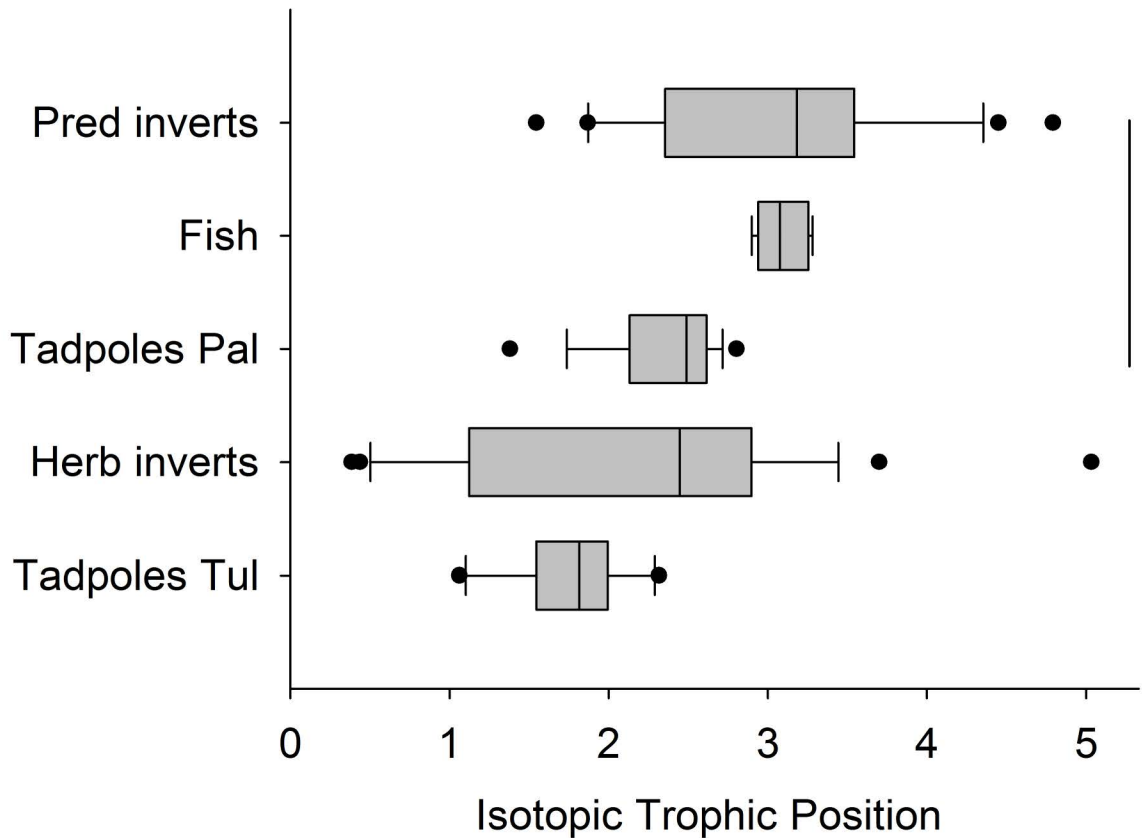
745

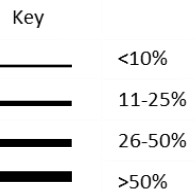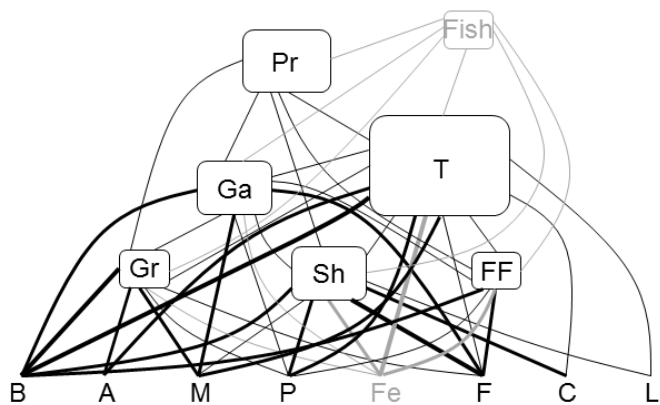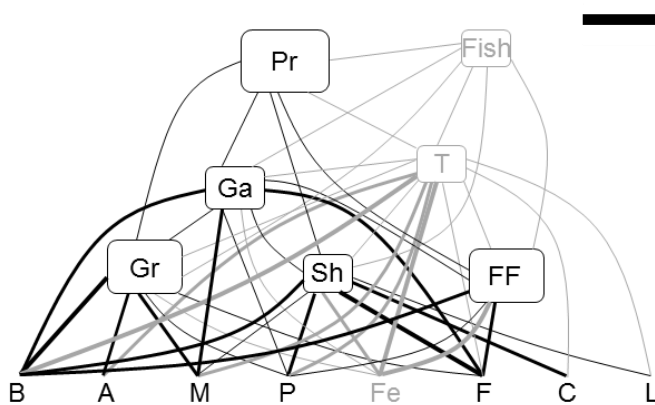746   Table S5. Assignment of invertebrates to consumer feeding groups.
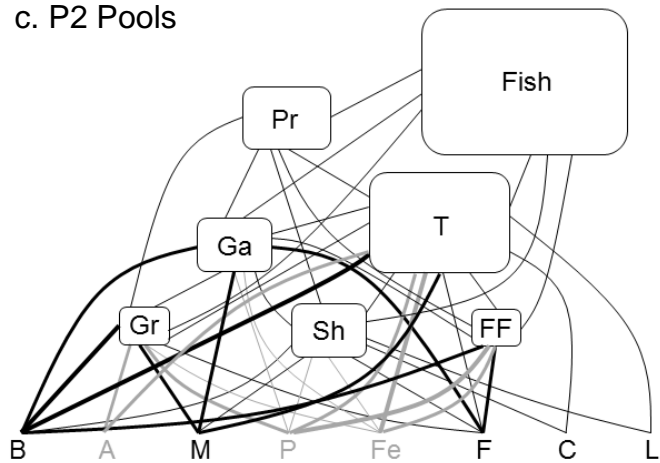
a. Paluma

b. Tully

Isotopic Trophic Position

a. P1 Pools

b. P1 Riffles

c. P2 Pools

d. P2 Riffles
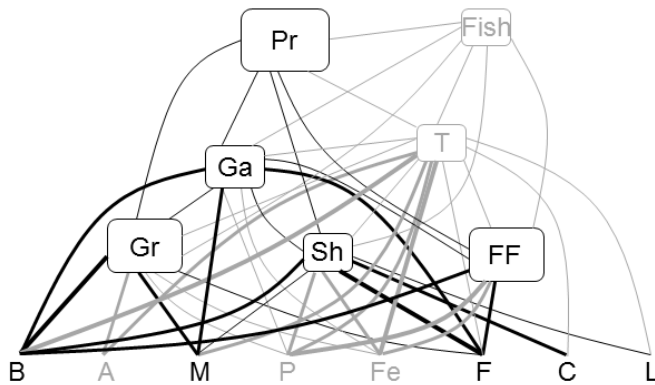
e. P3 Pools

f. P3 Riffles

Key

<10%

11-25%

26-50%

>50%
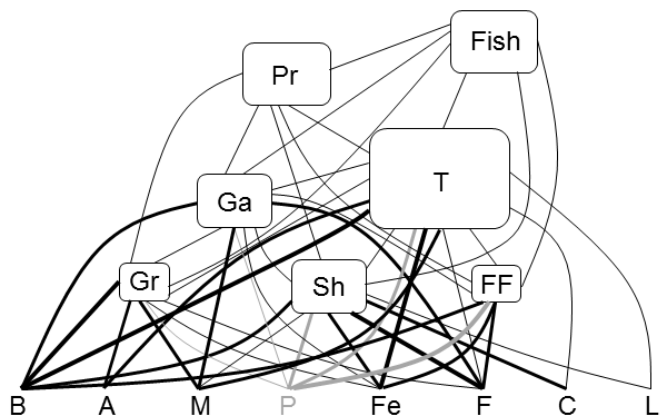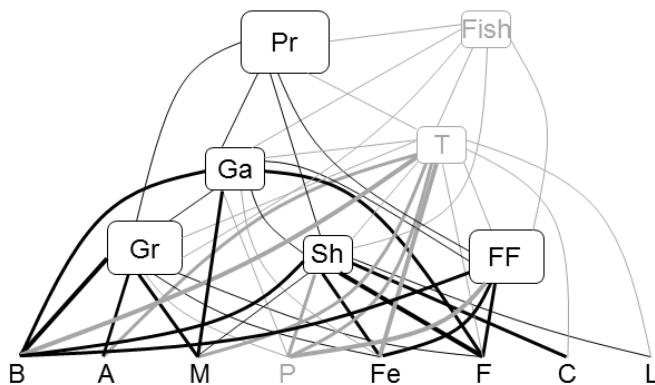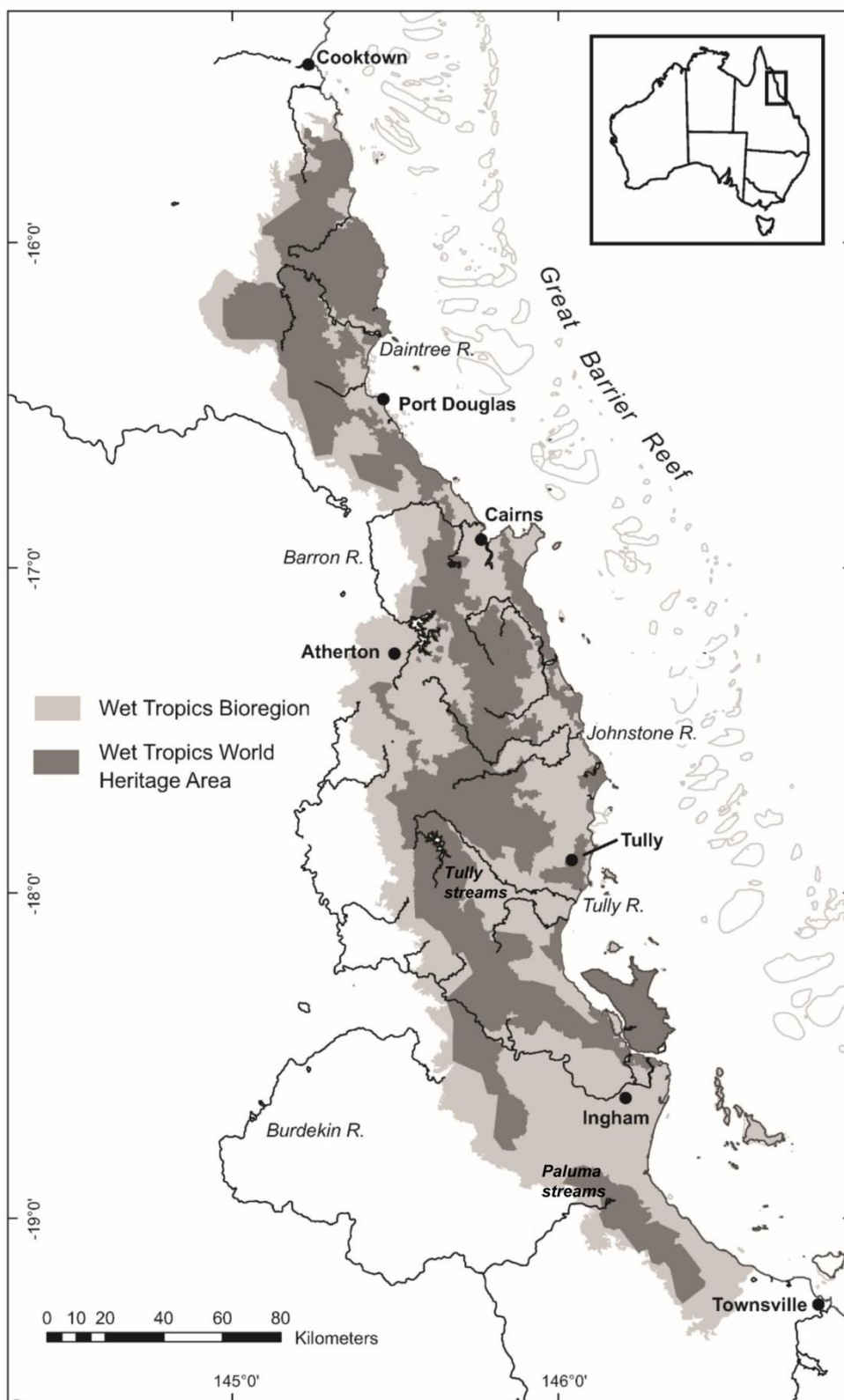
Figure S1. Location of study streams in the Queensland Wet Tropics. Map drawn by Adella Edwards.

Figure S2. Mean (± s.d.) $\delta^{15}N$ and $\delta^{13}C$ ratios of tadpoles at (a) Paluma in 2012 and 2013, and (b) Tully in 2013. Each point represents a specific size class of a species. Species codes: Ld = *Litoria dayi,* Ln = *L. nannotis,* Lr = *L. rheocola,* Ls = *L. serrata,* Mc = *Mixophyes coggeri.* Size classes: 1 = large, 2 = medium, 3 = small, 4 = small and medium tadpoles combined.

Figure S3. Mean (± s.d.) δ15N and δ13C ratios of invertebrate herbivores and predators at Paluma in 2012. Each point represents a taxon of a specific feeding group. Feeding group codes for herbivores: FF = filterer, Ga = gatherer, Gr = grazer and Sh = shredder. See Table 2 for invertebrate taxa identified and grouped according to feeding mode.

Table S1. Basal sources present in the streams at Paluma and Tully. Sources were combined (indicated by hyphenation) when the $\delta^{13}C$ measures differed by less than 1.0 ‰.

Area	Stream reach	Basal sources
Paluma	P1	Filamentous algae, moss, leaves, biofilm-periphyton, CPOM-FPOM
	P2	Biofilm, moss, leaves-CPOM-FPOM
	P3	Biofilm, filamentous algae, moss, iron matrix, leaves-CPOM-FPOM
Tully	T1	Biofilm, filamentous algae, leaves-FPOM
	T2	Biofilm, filamentous algae, leaves-FPOM

Table S2. The C:N ratio ± SD of basal sources in the streams at Paluma and Tully. A lower C:N ratio indicates a higher nutrient quality. For basal sources that were collected in only one riffle or pool in a stream site, the standard deviations were calculated using data from all the riffles and pools sampled within an area (except for the iron matrix, which was found in only one pool at Paluma). Differences between overall ratios indicated by different letters under TUKEY (Tukey test, $P < 0.05$, following ANOVA, $F_{7,75} = 50.83$, $P < 0.0001$).

	PALUMA			TULLY		TUKEY
Basal source	P1	P2	P3	T1	T2	
Leaves	44.5 ± 10.3	53.0 ± 15.3	45.2 ± 6.2	68.7 ± 9.3	53.9 ± 11.1	a
CPOM	31.4 ± 1.2	31.8 ± 1.5	33.0 ± 1.4	-	-	b
FPOM	25.5 ± 1.0	25.2 ± 0.5	25.6 ± 0.9	20.3 ± 0.7	18.8 ± 1.1	b
Moss	22.5 ± 5.7	25.0 ± 1.2	28.2 ± 2.8	-	-	b
Periphyton	27.8 ± 12.3	-	-	-	-	bc
Iron matrix	-	-	19.6	-	-	bc
Biofilm	12.5 ± 2.0	10.5 ± 2.7	10.2 ± 4.3	10.6 ± 0.2	10.3 ± 0.2	c
Filamentous algae	9.2 ± 0.5	-	8.9 ± 0.9	11.6 ± 0.6	7.8 ± 1.7	c

Table S3. Isotopic trophic positions (ITPs) for tadpoles at Paluma in 2012 and 2013, and Tully in 2013. The tadpoles were categorised as primary consumers if ITP was close to 2 (1.9 - 2.1), as secondary consumers if ITP was close to 3 (2.9 – 3.1), and as omnivores if ITPs were not centred on an integer. Tadpole size classes were: small (< 10 mm), medium (10 to < 22 mm) and large (≥ 22 mm) for *Mixophyes coggeri* tadpoles, and small (< 7.5 mm), medium (7.5 to < 12 mm) and large (≥ 12 mm) for other species.

Species	Size class	PALUMA						TULLY	
		P1		P2		P3		T1	T2
		2012	2013	2012	2013	2012	2013	2013	2013
L. serrata	Large	-	-	2.5	-	-	-	1.8	-
	Medium	2.8	2.0	2.4	2.1	2.6	2.6	-	-
	Small	-	-	2.3	1.9	-	-	-	-
	Small/ medium	-	-	-	-	2.5	1.4	-	1.1
M. coggeri	Large	2.7	-	2.6	-	2.5	-	-	-
	Medium	2.5	-	2.5*	-		-	-	-
L. nannotis	Large	-	-	-	-	-	-	1.8	1.6
	Small/ medium	-	-	-	-	-	-	2.1	-
L. rheocola	Large	-	-	-	-	-	-	1.7	-
	Medium	-	-	-	-	-	-	1.5	-
	Small	-	-	-	-	-	-	1.8**	
L. dayi	Large	-	-	-	-	-	-	2.0	-
	Small/ medium	-	-	-	-	-	-	2.3	-

* Sample from P2 and P3

** Sample from T1 and T2

Table S4. Isotopic trophic positions (ITPs) for invertebrates and fishes at Paluma in 2012. Invertebrates were categorised as primary consumers if ITP was close to 2 (1.9 - 2.1), as secondary consumers if ITP was close to 3 (2.9 – 3.1), and as omnivores if ITPs were not centred on an integer.

Taxon	Family	Feeding group	P1	P2	P3
Diptera	Simuliidae	Filterer	2.5	-	1.8
	Mixed	Filterer	-	0.7	-
"Several"	Mixed	Gatherer/filterer	2.6	2.0	-
Parastacidae	Large	Gatherer	1.9	2.5	2.5
	Medium	Gatherer	3.4	2.7	2.8
	Small	Gatherer/predator	3.1	3.2	3.0
Palaemonidae	Medium	Grazer	-	-	5.0
Coleoptera	Psephenidae	Grazer	3.7	2.3	-
Ephemeroptera	Leptophlebiidae	Grazer/shredder/gatherer	0.4	1.0	0.8
	Mixed	Grazer	1.3	2.4	2.2
Plecoptera	Gripopterygidae	Grazers	-	2.5	3.1
Trichoptera	Mixed	Grazer/gatherer/filterer	3.2	-	-
	Philopotamidae	Grazer/gatherer/filterer	-	2.5	-
	Mixed	Shredder	0.9	1.5	0.4
Lepidoptera		Grazer/shredder	-	0.5	-
"Several"	Mixed	Predator	4.3	3.0	-
Coleoptera	Dytiscidae	Predator	-	1.9	2.0
	Mixed	Predator	1.5	-	-
Ephemeroptera	Ameletopsidae	Predator	3.3	3.3	-
Plecoptera	Mixed	Predator/grazers	3.3	-	-
Hemiptera	Gelastocoridae	Predator	-	1.9	-
	Mixed	Predator	2.3	3.1	1.9
Megaloptera	Corydalidae	Predator	2.8	-	3.1
Arachnida	Pisauridae	Predator	2.4	-	3.3
Zygoptera	Synlestidae	Predator	4.8	3.6	-
	Mixed	Predator	4.4	3.5	3.9
Epiproctophora	Gomphidae	Predator	-	1.9	-
	Synthemistidae	Predator	-	3.0	-
	Telephlebiidae	Predator	-	3.4	-
	Mixed	Predator	3.3	-	3.5
Trichoptera	Mixed	Predator	4.1	3.6	-
Fishes	*Mogurnda adspersa*	Predator	-	-	3.2
	Anguilla reinhardtii	Predator	-	3.0	-

Table S5: Assignment of invertebrates to consumer feeding groups (according to Merritt & Cummins, 1984; Gooderham & Tsyrlin, 2002; Cheshire, Boyero & Pearson, 2005), including assignment to broader mixed groups (right column) for when invertebrates were combined for stable isotope analysis.

Consumer feeding group	Filterer	Gatherer	Grazer	Shredder	Predator	Combined invertebrate groups
Ameletopsidae	.	.	.	.	●	.
Athericidae	.	.	.	.	●	Predators mixed
Baetidae	.	●	●	.	.	Ephemeroptera grazers mixed
Caenidae	.	●	●	.	.	Ephemeroptera grazers mixed
Calamoceratidae	.	.	.	.	.	
Anisocentropus sp.	.	.	.	●	.	Trichoptera shredders mixed
Ceratopogonidae	.	●	.	.	●	Predators mixed
Chironomidae	●	●	.	.	.	Gatherers/filterers mixed
Chorismagrionidae	.	.	.	.	●	Zygoptera predators mixed
Cordulephyidae	.	.	.	.	●	Epiproctophora predators mixed
Corydalidae	.	.	.	.	●	
Culicidae	●	.	.	.	.	Diptera filterers mixed
Diphlebiidae	.	.	.	.	●	Zygoptera predators mixed
Dixidae	●	.	.	.	.	Diptera filterers mixed
Dolichopodidae	.	.	.	.	●	Predators mixed
Dytiscidae	.	.	.	.	●	
Ecnomidae	.	.	.	.	●	Trichoptera predators mixed
Eustheniidae	.	.	.	.	●	Plecoptera predators/grazers mixed
Gelastocoridae	.	.	.	.	●	
Gerridae	.	.	.	.	●	Hemiptera predators mixed
Gomphidae	.	.	.	.	●	
Gripopterygidae	.	.	●	.	.	
Gyrinidae	.	.	.	.	●	Coleoptera predators mixed
Helicopsychidae	.	.	●	.	.	Trichoptera grazers/gatherers/filterers mixed
Hydrobiosidae	.	.	.	.	●	Trichoptera mixed predators
Hydrometridae	.	.	.	.	●	Hemiptera predators mixed
Hydropsychidae	●	.	.	.	.	Trichoptera grazers/gatherers/filterers mixed
Hypolestidae	.	.	.	.	●	Zygoptera predators mixed
Lepidoptera	.	.	●	●	.	
Leptoceridae	.	.	.	.	.	
Lectrides sp.	.	.	.	●	.	Trichoptera shredder mixed
Oecetis sp.	.	.	.	●	.	Trichoptera shredder mixed
Triplectides sp.	.	.	.	●	.	Trichoptera shredder mixed
Triplexa sp.	.	.	.	●	.	Trichoptera shredder mixed
Leptophlebiidae	.	.	.	.	.	
Atalophlebia sp.	.	●	●	●	.	Ephemeroptera grazers/gatherers/ shredders mixed
Genus Q	.	●	●	●	.	Ephemeroptera grazers/gatherers/ shredders mixed
Jappa sp.	.	●	●	●	.	Ephemeroptera grazers/gatherers/ shredders mixed

Consumer feeding group	Filterer	Gatherer	Grazer	Shredder	Predator	Combined invertebrate groups
Leptophlebiidae	.	.	.	.	.	
Koornonga sp.	.	•	•	•	.	Ephemeroptera grazers/gatherers/ shredders mixed
Neboissophlebia sp.	.	•	•	•	.	Ephemeroptera grazers/gatherers/ shredders mixed
Nousia sp.	.	•	•	•	.	Ephemeroptera grazers/gatherers/ shredders mixed
Leptophlebia sp.	.	•	•	•	.	Ephemeroptera grazers/gatherers/ shredders mixed
Ulmerophlebia sp.	.	•	•	•	.	Ephemeroptera grazers/gatherers/ shredders mixed
WT sp 2/4	.	•	•	•	.	Ephemeroptera grazers/gatherers/ shredders mixed
Lindeniidae	.	.	.	.	•	Epiproctophora predators mixed
Nevrorthidae	.	.	.	.	•	Predators mixed
Notonectidae	.	.	.	.	•	Hemiptera predators mixed
Oxygastridae	.	.	.	.	•	Epiproctophora predators mixed
Parastacidae	.	.	.	.	.	
large	.	•	.	.	.	
medium	.	•	.	.	.	
small	.	•	.	.	•	
Philopotamidae	•	•	•	.	.	
Pisauridae	.	.	.	.	•	
Pleidae	.	.	.	.	•	Hemiptera predators mixed
Polycentropodidae	.	.	.	.	•	Trichoptera mixed predators
Psephenidae	.	.	•	.	.	
Pseudocorduliidae	.	.	.	.	•	Epiproctophora predators mixed
Scirtidae	•	.	.	.	.	Gatherers/filterers mixed
Simuliidae	•	.	.	.	.	
Stratiomyidae	.	•	.	.	.	Gatherers/filterers mixed
Synlestidae	.	.	.	.	•	
Synthemistidae	.	.	.	.	•	
Telephlebiidae	.	.	.	.	•	
Veliidae	.	.	.	.	•	Hemiptera predators mixed