

1 Carbon sources supporting Australia's most widely distributed freshwater fish, *Nematalosa erebi*
2 (Günther) (Clupeidae: Dorosomatinae)

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36 **Abstract**

37 Both brown (detrital-based) and green (algal-based) food pathways support freshwater food webs,
38 although the importance of either source may vary within species, regions and different phases of the
39 flow regime. The bony bream (*Nematalosa erebi* Clupeidae: Dorosomatinae) is one of Australia's
40 most widely distributed freshwater fish species and is a key component of freshwater food webs,
41 especially in northern Australia. We sought to better define the feeding habits of this species,
42 previously classified as a detritivore, algivore or zooplanktivore (or combinations thereof) by
43 undertaking meta-analyses of published accounts based on stomach content analysis and ^{13}C and ^{15}N
44 stable isotope analysis. Stomach content analysis clearly indicated that detritus was the dominant
45 food item although benthic algae could be an important dietary component in some habitats (inland
46 river floodplains) and during the wet season. Zooplankton were important for small fish (i.e.
47 juveniles < 100 mm in length). SIA, when data were pooled across a large number of locations,
48 indicated that detritus derived from terrestrial vegetation was better aligned isotopically with values
49 for both adult and juvenile bony bream, while algae were comparatively ^{13}C -enriched, indicating the
50 latter source was not the dominant contributor to the biomass of this species. However, using site
51 specific data and a regression approach, a significant relationship between algal carbon and that of
52 large fish was revealed, suggesting that carbon derived from benthic algae contributed about 20% of
53 the carbon of adult bony bream. Zooplankton contributed a similar amount. Zooplankton provided
54 the majority of carbon for small fish. We contend that detritus derived from terrestrial vegetation is
55 the likely remaining carbon source for large bony bream and this interpretation was supported by the
56 outcomes of multiple regression analyses. Although previous studies of aquatic food webs in northern
57 Australia have emphasized the importance of high quality algal basal resources, this study indicates
58 that terrestrial sources may be important for some species and demonstrates the need to better
59 consider the circumstances which cause biota to switch between different food sources.

60 **Introduction**

61 Most plant matter ends up as detritus and most community food webs contain both detrital
62 and living primary producer energy channels (brown and green food chains, respectively) (Moore et
63 al. 2004, Rooney et al. 2006). Early models of aquatic ecosystem function emphasised the importance
64 of terrestrial or aquatic vascular plant material in supporting the biomass of aquatic consumers via a
65 detrital breakdown pathway (Vannote et al. 1980; Junk et al. 1989). Qualification of this viewpoint
66 has included the inclusion of microbiota as both conditioners of detritus that make nutrients and
67 energy more available and as constituents which are themselves consumed (e.g. France 2011). In
68 contrast, while not discounting the importance of terrestrial inputs, Thorp and Delong (1994)
69 emphasised the importance of algal production in supporting consumer biomass. The use of stable
70 isotopes and fatty acid markers in food web studies has largely confirmed the importance of
71 autochthonous algal production in aquatic food webs (Lewis et al. 2001; Bunn et al. 2003; Guo et al.
72 2016 a, b; Brett et al. 2017). Algal carbon is easier to digest and assimilate than that of vascular plant
73 material (Brett et al. 2017). Moreover, algae contain higher quantities of polyunsaturated fatty acids
74 (PUFA) which are essential for metazoan growth (Guo et al. 2016a).

75 Douglas et al. (2005) proposed that most biomass of tropical northern Australian rivers was
76 ultimately derived from algal production. This hypothesis is largely supported by subsequent
77 research, although other sources, such as terrestrially-derived detritus, may be important also (Bunn et
78 al. 2013; Pettit et al. 2017). Elsewhere, several experimental and field-based studies have revealed
79 that some aquatic consumers are supported by carbon derived from detritus and attached microbes
80 (e.g. McGoldrick et al. 2008; Reid et al. 2008; Brett et al. 2009; Solomon et al. 2011; Belicka et al.
81 2012). Further, fatty acid profiles of some primary consumer organisms indicate a detrital origin via
82 microbial processors (Belicka et al. 2012) and some aquatic organisms may possess the capacity to
83 convert some fatty acids into more physiologically-active forms (Murray et al. 2014; Guo et al.
84 2016b). Brett et al. (2017) suggest that the extent to which terrestrial carbon supported upper trophic
85 level production may depend on the simultaneous availability of essential biomolecules derived from
86 algae and concluded that there is no doubt that terrestrially-derived carbon is ingested and assimilated

87 by herbivores but that it is done so at much reduced efficiency. Clearly, an algal/detrital dichotomy
88 oversimplifies the complex relationships present within aquatic food webs (Taylor and Batzer 2010,
89 Jardine et al. 2015).

90 Detritivorous fishes are an important component of tropical aquatic food webs (Lowe-
91 McConnell 1975; Goulding et al. 1988; Flecker 1996), transferring basal production to higher trophic
92 levels and frequently forming the major prey of piscivorous fishes of socio-economic importance
93 (Winemiller 2004). Whilst detritivorous fishes are common in tropical regions globally, detritivory is
94 less evident in temperate regions (Egan et al. 2018). Coates (1993) stated that truly detritivorous
95 fishes are absent from freshwaters of the Australasian region, tropical or otherwise, but subsequent
96 studies have revealed that detritus may comprise a significant fraction of the stomach contents of
97 several benthic foraging species particularly in tropical northern Australia (Pusey et al. 2000; Bishop
98 et al. 2001; Kennard et al. 2001).

99 *Nematalosa erebi* (Günther) (bony bream), a clupeid gizzard shad, is one of Australia's most
100 widely distributed freshwater fish species. It has been previously characterised as a detritivore,
101 algivore or planktivore (or combinations thereof) (see Pusey et al. (2004) for a review of biology).
102 This species is primarily tropical/subtropical although its distribution extends as far south as 35°S in
103 the Murray-Darling River system (Figure 1). It occurs in a great variety of perennial and intermittent
104 aquatic habitat types and may achieve very high levels of abundance. *Nematalosa erebi* is itself
105 consumed by many higher order consumers including piscivorous fishes, crocodiles and birds such as
106 cormorants and pelicans; it is thus an important component in the food webs of Australian rivers,
107 particularly those of northern Australia.

108 We sought to better define the feeding habits and role of *N. erebi* in aquatic food webs by
109 reference to published dietary information and more recent stable isotope analysis of food web
110 structure. We specifically sought to determine whether it was reliant on allochthonous production
111 (i.e. terrestrial vegetation via a detrital pathway) or autochthonous algal production. We hypothesised
112 that algae were the main source of carbon for this species.

113 **Materials and methods**

114 *Sources of dietary information – stomach content analysis*

115 Dietary information for *N. erebi* was available from 17 individual studies drawn from the
116 published literature, a University thesis, two consultancy reports to State government and one
117 unpublished dataset (mean sample size = 191.5 ± 61.6 (SE)) (Figure 1). These data were drawn from
118 a larger dataset being used to examine the trophic ecology of Australian freshwater fishes in which
119 diet was apportioned to proportional contributions within 15 categories (see Kennard et al. (2001) for
120 further details concerning diet summarization and data treatment). Here we present information for
121 only five categories (AQINS – aquatic insects, MAC – aquatic macrophytes, DET – detritus, ALG –
122 algae (including filamentous algae, diatoms and biofilm) and ZOOPL – zooplankton) as these
123 collectively accounted for $96.5\% \pm 1.8$ (SE) of the diet across studies (Table 1). We also included
124 some measure of size of the fish examined in each study either as mean size (\pm SE) or range in size.
125 The lower limit of the size range of individuals included was >100 mm SL for eight studies (i.e.
126 adults), whereas four studies were largely dominated by individuals < 100 mm SL (i.e. juveniles).
127 These latter studies were undertaken within the Murray-Darling Basin, with the exception of one
128 undertaken in the Burdekin River of Central North Queensland (Figure 1). Most studies attempted to
129 distinguish between detritus *per se* and microalgae mixed in with small detrital particles. Only four
130 of the 17 reviewed studies included data from both wet season and dry season sampling periods. Dry
131 season samples were collected as part of a one-off sampling event.

132 *Sources of stable isotope information*

133 We sourced information on carbon and nitrogen stable isotope (SI) values of bony bream
134 tissue (fin or muscle) and three potential food sources (benthic algae (primarily periphyton), terrestrial
135 vegetation and zooplankton) from 11 separate food web studies undertaken in northern, eastern and
136 central Australia and the northern portion of the Murray Darling Basin (Figure 1 and Supplementary
137 Information Table 1) in which the authors have been individually or collectively involved and which
138 included *N. erebi*. These studies were intended to examine nutrient and energy transfer between a

139 variety of basal sources, many organisms (including many species of fish) and trophic levels. Only
140 three of these studies were undertaken in the dry season only. We excluded any data that did not
141 allow us to distinguish between fish of different size classes (i.e. <100 mm Standard Length (SL) and
142 >100 mm SL). The manner in which samples were collected and analysed was largely consistent
143 across studies (see detailed methods in Jardine et al. 2012a) with the exception of particulate organic
144 matter for which different particle sizes (coarse - CPOM and fine - FPOM) were not consistently
145 differentiated nor collected. In contrast, all studies collected dead leaves of riparian trees (TVEG -
146 primarily *Melaleuca* and *Eucalyptus* spp.) and these species contribute most allochthonous carbon
147 inputs to freshwater systems in the study area. For those samples in which SI information was
148 available for TVEG, CPOM and FPOM, $\delta_{13}\text{C}$ values of CPOM and FPOM differed from TVEG by
149 <+1‰ and +2-3‰, respectively, and differences in ‰ $\delta_{15}\text{N}$ were <+1‰. These differences accord
150 well with similar comparisons elsewhere (e.g. Hedges et al 1994; Finlay and Kendall 2007). In total,
151 SI information was available for fish collected from 120 separate locations (i.e. sites). $\delta_{13}\text{C}$ and $\delta_{15}\text{N}$
152 values for putative source material for each site were estimates based on the mean of at least three
153 samples. Similarly, information from at least three, but often up to 20 individuals, for each size class
154 of *N. erebi* was used to estimate mean $\delta_{13}\text{C}$ and $\delta_{15}\text{N}$ values of fish at each site.

155 We generated histograms of the frequency distributions for $\delta_{13}\text{C}$ and $\delta_{15}\text{N}$ for the three food
156 sources and both size classes of *N. erebi* across all sites to assess the extent of spatial variation in
157 isotopic values and the extent of overlap in isotopic values for different potential food sources. Broad
158 distributions (i.e. high variance) indicate high spatial variation. We also assessed whether $\delta_{13}\text{C}$ or
159 $\delta_{15}\text{N}$ of individual source materials varied independently using Pearson's correlation. Gradient-based
160 approaches where isotopic variation of producers and consumers is measured at multiple locations
161 have proved useful for determining the importance of different food sources exhibiting large spatial
162 variation in isotopic values (Rasmussen 2010, Jardine et al. 2012a). This approach, in contrast to a
163 mixing model approach, does not require potential sources to be distinct at all sites, does not require *a*
164 *priori* knowledge of the extent of isotopic trophic discrimination, and does not require spatial
165 variation in isotopic values of different consumers to be independent (Moore and Stevens 2008;

166 Rasmussen 2010). From a practical viewpoint, a gradient-based approach can maximise the number
167 of locations used in analyses as it does not require all three potential sources of carbon to have been
168 measured at every site.

169 Such an approach is well-suited to the present case where data were collected from multiple
170 locations within many rivers. We plotted $\delta_{13}\text{C}$ values of each size class of *N. erebi* against $\delta_{13}\text{C}$
171 values of algae, terrestrial vegetation and zooplankton. We used simple linear regression to assess the
172 strength of the relationship between consumer (i.e. *N. erebi*) $\delta_{13}\text{C}$ values and food source (i.e. algae,
173 terrestrial vegetation and zooplankton) $\delta_{13}\text{C}$ values and report statistical significance at the $\alpha = 0.05$
174 level. We estimated whether the slopes of the relationship between isotopic values were significantly
175 different from one or zero (i.e. not within the 95% confidence limits of the estimated slope). A close
176 dependency on one source or the other should see *N. erebi* muscle $\delta_{13}\text{C}$ values aligned with spatial
177 variation in $\delta_{13}\text{C}$ for that potential source (i.e. values should fall along a line denoting a 1:1
178 relationship or slope = 1). Conversely, if no significant relationship (i.e. slope = 0) is detected
179 between consumer and food sources isotopic values, then it is assumed that source is unlikely to be
180 important. Slopes significantly different from both 0 or 1 indicate a mixed feeding model (i.e. more
181 than one source contributes to the carbon or nitrogen assimilated into body mass) (Jardine et al.
182 2012a). An important assumption in this approach is that isotopic ratios in the consumer organism are
183 in equilibrium with the measured source materials; i.e. isotopic values do not reflect feeding at some
184 other time or location. To a large degree, this assumption underpins most food web studies
185 undertaken in natural environments. We similarly examined and tested the relationship between $\delta_{15}\text{N}$
186 values of potential food sources and those of *N. erebi* tissue. We also performed multiple regression
187 analyses for each size class and both isotopes for those locations for which information concerning all
188 three potential sources was available ($\delta_{13}\text{C}$: $n = 29$ and 35 for small and large fish, respectively; $\delta_{15}\text{N}$:
189 $n = 28$ and 29 for small and large fish, respectively), Finally, we estimated $\delta_{15}\text{N}$ trophic enrichment
190 factors (i.e. $\Delta\delta_{15}\text{N} = \delta_{15}\text{N}_{\text{consumer}} - \delta_{15}\text{N}_{\text{source}}$) for each source and age class combination and from these
191 data estimated overall trophic enrichment taking into account the estimated proportional contribution
192 by each source derived from the slopes of the lines relating $\delta_{13}\text{C}$ variation of source and consumers.

193 **Results**

194 *Stomach contents analysis*

195 Across all studies, the average contribution of detritus to the diet was $63\% \pm 7.6$ (SE) and that
196 of algae $20.8\% \pm 9.0$ (Table 1). Zooplankton contributed a further $9\% \pm 4.2$ and aquatic insect larvae
197 and aquatic macrophytes formed only a minor fraction of the diet (2.5 and 1.1%, respectively).
198 Detritus was the dominant dietary component in most studies except two undertaken in arid zone
199 rivers where algae contributed 90% and 52% to the diet (studies 10 and 11, respectively) and another
200 undertaken in the Gulf Cape York Peninsula region (study 4) in which detritus and algae were co-
201 dominant. Consumption of zooplankton was greatest in arid zone or southern regions (i.e. MDB);
202 however, high consumption of zooplankton was recorded in northern regions also (i.e. Kimberley and
203 North Australia). All studies in which zooplankton contributed more than 1% of the diet (seven
204 studies) were either dominated by, or included, fish less than 100 mm SL. For example, zooplankton
205 comprised 87.3% of the diet in a seasonal subsample comprised entirely of small fish within one study
206 undertaken in the Kimberley region (study 8). Similarly, a high contribution of zooplankton was
207 recorded in the most southern study available (MDB study 17) and for which the sample was also
208 dominated by small individuals. Aquatic insect larvae (chironomid larvae) comprised ~10% of the
209 diet in another study (study 9) undertaken in a large shallow sandbed river. Individuals included in
210 that study were also small (mean SL = 70 ± 2 (SE) mm). Thus, consumption of zooplankton and, to a
211 lesser extent, aquatic insect larvae, was limited to individuals of small size. Consumption of detritus
212 and algae was greatest in larger individuals (i.e. >100 mm SL).

213 *Stable isotope analyses*

214 A wide range of $\delta_{13}\text{C}$ values from -34.6 to -12.0‰ and a mean (\pm SE) of $-23.0 \pm 0.5\%$ was
215 recorded for benthic algae (Figure 2). Terrestrial vegetation was relatively depleted in ^{13}C and varied
216 little (mean $\delta_{13}\text{C} = -29.1 \pm 0.2\%$, range = -33.3 to -26.5‰). Zooplankton $\delta_{13}\text{C}$ values ranged from -
217 38.2 to -23.5‰ and were typically highly depleted in ^{13}C (mean $\delta_{13}\text{C} = -31.1 \pm 0.4\%$). Large *N. erebi*
218 exhibited an intermediate range of $\delta_{13}\text{C}$ values (-33.3 to -18.5‰, mean $^{13}\text{C} = -27.4 \pm 0.3\%$) and were

219 more depleted in ^{13}C compared to algae (as were small *N. erebi*: range = -33.7 to -20.9‰; mean $\delta_{13}\text{C}$
220 = $-28.4 \pm 0.3\text{‰}$). $\delta_{13}\text{C}$ values of algae, terrestrial vegetation and zooplankton varied independently of
221 one another ($r < 0.20$, $p > 0.05$ for all comparisons).

222 Algae and terrestrial vegetation had similar means and variability in $\delta_{15}\text{N}$ values (mean $\delta_{15}\text{N}$ =
223 $4.5 \pm 0.3\text{‰}$, $4.3 \pm 0.2\text{‰}$, respectively) (Figure 2). Zooplankton, in contrast, were comparatively
224 enriched in ^{15}N (mean $\delta_{15}\text{N}$ = $9.2 \pm 0.5\text{‰}$) and some samples were highly enriched (maximum =
225 18.9‰). Large *N. erebi* were similarly enriched in ^{15}N (mean $\delta_{15}\text{N}$ = $9.0 \pm 0.2\text{‰}$) and small *N. erebi*
226 were slightly more enriched in ^{15}N than larger fish and zooplankton (mean $\delta_{15}\text{N}$ = $10.8 \pm 0.3\text{‰}$). $\delta_{15}\text{N}$
227 values of algae, terrestrial vegetation and zooplankton did not vary independently of one another (r =
228 0.60, 0.51 and 0.93, $p < 0.001$ for algae/terrestrial vegetation, algae/zooplankton and terrestrial
229 vegetation/zooplankton, respectively).

230 Figure 3 plots isotopic variation ($\delta_{13}\text{C}$ and $\delta_{15}\text{N}$) in large and small *N. erebi* against variation
231 in isotopic values of putative dietary components across a large number of sites (see Table 2 for
232 sample sizes and regression statistics). Variation in $\delta_{13}\text{C}$ of large *N. erebi* was significantly positively
233 related to variation in $\delta_{13}\text{C}$ of both algae and zooplankton and the slopes for these relationships (0.22
234 and 0.33, respectively) were both significantly different from 0 and 1, suggesting a mixed feeding
235 strategy with algae and zooplankton together accounting for about one half of assimilated carbon. No
236 significant relationship between $\delta_{13}\text{C}$ of large *N. erebi* and terrestrial carbon was detected. Variation
237 in $\delta_{13}\text{C}$ of small *N. erebi* was not significantly related to variation in benthic algal $\delta_{13}\text{C}$ values but was
238 significantly positively correlated with zooplankton $\delta_{13}\text{C}$, with the slope of this relationship (0.52)
239 being significantly different from 0 and 1 (Table 2); again suggesting a mixed feeding model with
240 about one half of assimilated carbon being derived from this source (Figure 3, Table 2).

241 $\delta_{15}\text{N}$ variation in both large and small *N. erebi* was significantly positively correlated with
242 variation in all putative food sources (i.e. algae, terrestrial vegetation and zooplankton), perhaps not
243 surprisingly given variation within sources was also significantly positively correlated. However,
244 $\delta_{15}\text{N}$ variation in large *N. erebi* was most strongly correlated with variation in $\delta_{15}\text{N}$ of terrestrial
245 vegetation (Table 2). Trophic enrichment ($\delta_{15}\text{N}_{\text{consumer}} - \delta_{15}\text{N}_{\text{diet}}$) in large *N. erebi* averaged $4.9 \pm 0.3\text{‰}$,

246 $6.0 \pm 0.3\text{‰}$ and $0.5 \pm 0.2\text{‰}$ and in small *N. erebi* averaged $6.2 \pm 0.4\text{‰}$, $6.7 \pm 0.4\text{‰}$ and $1.5 \pm 0.4\text{‰}$
247 for algae, terrestrial vegetation and zooplankton, respectively. However, when scaled for the
248 different proportional contributions by each putative food source, the estimated average trophic
249 enrichment for all sources was $4.1 \pm 0.3\text{‰}$ and $3.2 \pm 0.4\text{‰}$ for large and small *N. erebi*, respectively.
250 The results of multiple regression analyses based on data available from the reduced set of locations
251 (Table 3) strongly supported the outcomes of simple linear regression analysis. That is, $\delta_{13}\text{C}$ values of
252 small fish (<100 mm SL) were most strongly related to those of zooplankton whereas those of the
253 larger size class were most strongly related to terrestrial vegetation values and then benthic algae.
254 $\delta_{15}\text{N}$ isotope information was less informative than $\delta_{13}\text{C}$ (as was the case for simple linear regression)
255 in that only zooplankton exhibited a significant relationship with $\delta_{15}\text{N}$ values of small fish.

256 **Discussion**

257 Stomach content analysis from multiple studies indicate that *Nematalosa erebi* is
258 zooplanktivorous as a juvenile before transitioning to a primarily detrital diet with increasing size.
259 These ontogenetic changes in diet mirror similar changes observed in a closely related clupeid, the
260 American gizzard shad *Dorosoma cepedianum* (Smoot and Findlay 2010). When based on all
261 samples, stable isotope information also suggested that detritus derived from terrestrial vegetation,
262 and zooplankton, provided a large fraction of the assimilated carbon. On average, the $\delta_{13}\text{C}$ values of
263 both small and large *N. erebi* ($-28.5 \pm 0.3\text{‰}$ and $-27.4 \pm 0.3\text{‰}$, respectively) were very closely
264 aligned to that of terrestrial vegetation, depleted in ^{13}C with respect to algal $\delta_{13}\text{C}$ values ($-23.0 \pm$
265 0.5‰) and enriched in comparison to zooplankton ($-31.1 \pm 0.4\text{‰}$). Collectively, these data do not
266 support a significant contribution by benthic algae to carbon assimilation in large *N. erebi*. However,
267 regression analyses using site-specific data revealed a significant positive relationship between algal
268 carbon and that of large fish, suggesting that benthic algae may also form an important carbon source
269 for this size class of *N. erebi*.

270 Important methodological considerations for both stomach content and stable isotope analyses
271 must be considered before accepting the generality of these findings. First, most studies, particularly
272 those undertaken in northern Australia, examined diet during the dry season only whereas the

273 consumption of algae was greatest in those few studies undertaken over a long period and which
274 included either wet seasons or periods immediately following a wet season. Thus, the contribution of
275 algae to the diet of *N. erebi* could conceivably be higher than reported here. Second, trituration of
276 ingested material within the muscular gizzard renders most material to a fine paste and it is highly
277 likely, despite the best intentions of researchers, that algal material, other than filamentous algae, may
278 not be readily identifiable (i.e. distinguished from detritus) and reliably quantified when examined
279 macroscopically. Third, in aggregating stable isotope information across studies and locations, any
280 spatial variation in the relationship between algal isotope values and those of the consumer is likely
281 obscured. As a consequence, conclusions regarding the importance of detritus derived from terrestrial
282 vegetation and a minimal contribution by benthic algae warrant further scrutiny.

283 Indeed, isotopic values for the putative food sources of algae and zooplankton varied greatly
284 and the range of values overlapped substantially for all sources. Such large variation in $\delta_{13}\text{C}$ is not
285 unexpected given the range of different potential pathways by which carbon is available for uptake
286 (i.e. atmospheric versus dissolved) and the wide array of factors that influence carbon fractionation in
287 aquatic systems (Finlay 2004; Barnes et al. 2007). Similarly, baseline values of $\delta_{15}\text{N}$ vary
288 extensively in space due to variation in taxonomic composition of producers, isotopic distinction
289 between various sources (i.e. N_2 , NO_3 , NH_3) and variation in the efficiency in which they are utilised
290 (Akiyama et al. 1997). It is notable however, that $\delta_{15}\text{N}$ values of terrestrial vegetation (riparian
291 species primarily within Myrtaceae) and benthic algae in the present study were highly correlated
292 despite their taxonomic distinctiveness. This finding suggests that both derived their nitrogen from
293 the same source (i.e. that dissolved within the stream and/or groundwater).

294 Rather than being an impediment to interpreting relationships between sources and
295 consumers, the presence of spatial variation in algal and zooplankton $\delta_{13}\text{C}$ values assists in identifying
296 the source of carbon sustaining *N. erebi*. Our data suggest a mixed feeding strategy in both large and
297 small individuals. A significant relationship between $\delta_{13}\text{C}$ values of algae and large fish with a slope
298 (0.22) significantly different from both 0 and 1, indicate that algae contribute about one-quarter (range
299 as defined by CIs: 10-35%) of the carbon assimilated by this size class. Carbon derived from benthic

300 algal production is important in freshwater ecosystems globally (Roach 2013) including Australia, and
301 especially in northern regions and arid zones (Bunn et al. 2003, 2006; Douglas et al. 2005, Leigh et al.
302 2010, Jardine et al. 2012a,b). Our study suggests however that benthic algal carbon contributed little
303 to biomass of small *N. erebi*, which were contrastingly more reliant on zooplankton carbon (52%;
304 range: 26-77%). Zooplankton also contributed substantially to the biomass of large *N. erebi* (33%;
305 range: 11-54%). Stomach content analysis for small *N. erebi* also identified zooplankton as an
306 important dietary component. Medeiros and Arthington (2011) report a significant correlation
307 between spatial variation in $\delta^{13}\text{C}$ values for *N. erebi* (and other fish species) and zooplankton similarly
308 consistent with the findings of stomach content analysis (Medeiros and Arthington 2008). Further,
309 Jardine et al. (2015) found that zooplankton accounted for 50% of assimilated carbon in small *N.*
310 *erebi* (~ 1 gm), declining to 25% in fish as large as 500 gm. Phytoplankton are typically highly
311 depleted in ^{13}C (Vuorio et al. 2006) and are the most likely source of carbon for zooplankton in the
312 present study. Planktonic algal production, in contrast to benthic algal production, likely contributes
313 to carbon assimilation in small individuals via their consumption of zooplankton.

314 Thus, algae and zooplankton potentially contribute about one-half of the carbon assimilated
315 by large *N. erebi* whereas zooplankton contribute about one half of the carbon assimilated by small *N.*
316 *erebi*. What then accounts for the remaining fractions? Whereas spatial variation and correlation
317 between source and consumer isotopic values proved useful here for quantifying the contribution of
318 algae and zooplankton, the minimal spatial variation in $\delta^{13}\text{C}$ values of terrestrial vegetation provided
319 little scope for doing so. None-the-less, stomach content analysis clearly indicates that detritus is the
320 dominant food item and the near absence of potential food items other than zooplankton or algae in
321 stomach contents strongly suggests that we have not failed to consider or assess other potential
322 sources. Moreover, the multiple regression analysis strongly supported a significant contribution by
323 terrestrial vegetation to *N. erebi* biomass. Thus, it seems most parsimonious to suggest that terrestrial
324 detritus is indeed the missing source, despite the failure to detect a correlation between detrital $\delta^{13}\text{C}$
325 values and those of fish, and the apparent poor nutritional quality of this food source (Brett et al.

326 2017). In addition, dead phytoplankton which have entered the detrital pool may have also
327 contributed to the carbon assimilated by *N. erebi*.

328 It is rare for detritus not to have attached or embedded bacteria and fungi (Bowen 1987;
329 Findlay et al. 2002). Detrital $\delta^{13}\text{C}$ values do not change greatly with conditioning and thus the
330 isotopic value of detritus, and of the microbial community living upon it, reflect its source origin
331 (Finlay and Kendall 2007). As a result, $\delta^{13}\text{C}$ values alone are unlikely to differentiate between carbon
332 derived from detritus from that derived from microorganisms feeding upon that detritus. Given the
333 refractory nature of vascular plant detritus, its nutritive value may be derived mostly from these
334 attached organisms (France 2011) despite their low biomass relative to their substrate (Bowen 1987).
335 Smoot and Findlay (2010) showed that the ingesta of the closely-related, facultative detritivore
336 *Dorosoma cepedianum* contained eight times more low-density material and was nutritionally
337 enriched compared to the detrital/sediment material upon which it foraged. Moreover, the microbial
338 biomass in ingesta was seven times greater than sediment. Smoot and Findlay (2010) suggest this
339 living component of detritus was used as a food source by this species. A similar comparison has not
340 been performed for *N. erebi*. If, however, *N. erebi* possesses the same capacity to winnow detrital
341 particles of differing quality, then it is possible that assimilation of carbon and nitrogen derived from
342 microbiota feeding upon detritus is substantial. There is scant information on ^{15}N fractionation by
343 microorganisms, making difficult any interpretation of enrichment patterns in consumers of this form
344 of prey (Vanderklift and Ponsard 2003); however, the high availability of microbial biomass within
345 the detrital pool can exert a disproportionate influence on enrichment dynamics on higher order
346 consumers that feed from both brown and green food chains (Steffan et al. 2017). We estimated a
347 trophic enrichment of 4.1‰ and 3.2‰ for large and small *N. erebi*, respectively; these values are not
348 dissimilar to the ~3‰ per trophic level increase reported by Vander Zanden and Rasmussen (2001)
349 and Post (2002). Bunn et al. (2013) report a trophic enrichment of $3.9 \pm 1.4\%$ for a range of
350 Australasian herbivorous fishes.

351 Although the quality of the fine detrital fraction may not be as high as that of algae and
352 certainly not that of zooplankton, it is none-the-less an abundant food source. Moreover, if the higher

353 value microbial fraction can be separated from lesser quality larger fractions, then its value is
354 increased further. Fish faced with a diet of low or reduced quality, particularly of protein, can
355 compensate by increasing consumption rates to meet both energy and essential nutrient demand
356 provided the food source is not limiting, which is not usually the case for detritus. Notwithstanding
357 the constraint imposed by the absence of intestinal structures enabling the processing of algae or
358 detritus (e.g. the muscular gizzard is largely absent in fish smaller than 60mm length), switching
359 between algal, detrital and zooplankton sources to achieve a blended diet across green and brown food
360 chains may enable juvenile *N. erebi* greater than 60 mm in length to achieve and maintain high
361 growth and intake of essential nutrients such as limiting amino acids and PUFAs.

362 Our study has shown that detritus (with or without associated microbiota), algae and
363 zooplankton are all important sources of carbon and nutrients for this species. *Nematalosa erebi* is
364 almost ubiquitous across northern Australia and may dominate fish biomass (Pusey et al. 2017). It is
365 itself consumed by many higher order predators, some of which can move great distances, even across
366 catchment boundaries in the case of water birds (Kingsford et al. 2010). Thus, the contribution of
367 terrestrially-derived carbon to *N. erebi* biomass, albeit occurring with low efficiency, may be
368 translated up into higher trophic levels of aquatic food webs of northern Australia. Furthermore, the
369 liberation of nutrients due to mass mortality of *N. erebi* in dry season waterholes of arid zone rivers
370 contributes greatly to the production dynamics of dry-season waters (Burford et al. 2008).
371 *Nematalosa erebi* is clearly an important component of riverine food webs. Although not entirely
372 dependent on detritus as a food source, detritus is an important component of the diet of *N. erebi*, and
373 thus may contribute more to tropical/subtropical Australian aquatic food webs than previously
374 considered. Our knowledge of the biology of *N. erebi* is scant, particularly in regard to the
375 relationship between hydrological variation, reproduction and movement. Changes in flow regimes
376 and connectivity between parts of the riverine landscape arising from expansion of water resource use
377 in northern Australia (Douglas et al. 2011, Pettit et al. 2017) and which impact on production
378 dynamics of *N. erebi* have the potential to disrupt riverine food webs (Turschwell et al. 2019). This
379 study has shown that both detritus and algae are important sources of energy and nutrients for this

380 common species and hence for food web structure in general. Moreover, it supports the assertion by
381 Jardine et al. (2015) that a focus on an algal/detrital dichotomy is unhelpful and that a greater focus on
382 the circumstances in which species switch between different food sources would provide a better
383 appreciation of the way in which food webs are structured and how they may change in response to
384 changes in hydrology. Furthermore, a greater focus on the carbon sources supporting zooplankton
385 production is warranted as zooplankton are key to early life history development of *N. erebi* and
386 probably to that of most other freshwater fish species of the region.

387

388

389 **Lay summary**

390 Bony bream is a very widely distributed Australian fish species. The study sought to define whether
391 this species is supported by autochthonous (algae) or allochthonous (detritus derived from terrestrial
392 vegetation) primary production. Stomach content analyses indicated that adults of this species feed
393 primarily on detritus whereas juveniles fed primarily on zooplankton. Stable isotope analyses in
394 contrast suggested that zooplankton, detritus and algae are all important sources of carbon.

395 **Conflicts of interest.**

396 The authors declare no conflicts of interest.

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Table 1 The diet of *Nematalosa erebi* as described in individual studies. Regions are identified in Figure 1. Habitat abbreviations in parentheses are R = riverine habitat, FP = floodplain, L = lacustrine. Size abbreviations are SL = standard length, TL = total length and CFL = length at caudal fork. SE = standard error and sd = standard deviation. Studies listed are: 1. Bishop et al. (2001), 2. Pusey et al. (2000), 3. Kennard (1995), 4. Hurtle and Pearson (1990), 5. Pusey et al. (1995), 6. Rayner et al. (2010), 7. Morgan et al (2004), 8. Thorburn et al. (2014), 9. Pusey et al. (2010), 10. Davies (unpubl. data, 2000), 11. Balcombe et al. (2005), 12. Bluhdorn and Arthington (1994), 13. Arthington et al. (1992), 14. Medeiros and Arthington (2014), 15. Medeiros and Arthington (2008), 16. Sternberg et al. (2008), and 17. Atkins (1984). Unless cited in the text, references are listed in Supplementary Information.

Study	Region, habitat and season	Size range (mm)	n	AQIN S	MAC	DET	ALG	ZOOP	Total	Comments
1	NTH-AUS, (R, FP) All seasonal flow phases	seasonal means ranged from 76 to 216, overall mean 120 LCF for entire sample	471	2.5	0.0	42.9	41.8	12.6	99.8	distinguished between detritus, desmids/diatoms and other algae
2	GOC-CYP (R) Dry season	120-270 SL	20	5.0	0.0	95.0	0.0	0.0	100.0	distinguished between detritus, desmids/diatoms and alga
3	GOC-CYP (FP) Dry season	200 (\pm 7 SE) and 178 (\pm 7 SL) for early and late dry season, resp.	98	0.1	0.0	99.1	0.6	0.1	99.9	distinguished between detritus and alga
4	GOC-CYP (R) Dry season	187-262 TL	7	0.0	0.0	50.0	50.0	0.0	100.0	distinguished between detritus and alga
5	WT (R) Dry season	200-300 SL	7	0.0	0.0	100.0	0.0	0.0	100.0	distinguished between detritus, desmids/diatoms and alga
6	WT (R) Wet and dry season	209 (\pm 18 SE) SL	66	0.1	0.2	97.2	0.8	0.2	98.4	distinguished between detritus and alga
7	SEQ-NEN (R)	>100	88	0.0	0.0	76.3	23.0	0.0	99.2	

	Dry Season										
8	KIMB (L) Dry season	47-330 TL	21	7.3	0.0	59.4	4.1	2.0	72.8	distinguished between biofilm/silt and filamentous algae. Tables list contribution by biofilm/silt but summary figures and text refer this component to detritus. Other plant material contributed 23.4% of diet	
9	KIMB (R) all flow phases	25-420 TL	132	0.3	0.0	58.6	15.7	12.9	87.2	distinguished between biofilm/silt and filamentous algae. Sand = 26.4% of diet in late dry and 9.7% of overall total diet (not included here). Tables list contribution by biofilm/silt but summary figures and text refer this component to detritus	
	Wet season	<100	11			97.7	1.6				
		>100	22			86.1	9.2				
	Early dry	<100	19					87.3			
		>100	36			68.0	24.0				
	Late dry	>100	44			48.7	21.4				
10	CNQ (R) Dry season	70 (2) SL	508	10.3	0.0	86.0	1.3	0.0	97.6	distinguished between detritus and alga	
11	PILB (R) Dry season	NA	9	6.0	0.0	0.0	92.0	0.0	98.0		
12	CENT AUS (WH, FP) Wet and dry season	29-260, majority <100mm TL	98	3.0	3.2	16.8	52.2	23.9	99.2		
13	SEQ-NEN (R) Dry season	>100	411	8.0	6.7	40.1	13.4	20.1	88.3		
14	MDB (WH) Dry season	67 (± 7 sd) - 100 (± 16 sd)	948	0.0	0.9	93.8	0.4	4.9	100.0	distinguished between detritus, filamentous alga, <i>Volvox</i> and "algal matter"	
15	MDB (WH) Dry season	72% of sample <69 SL	212	0.5	8.1	69.0	12.3	10.1	100.0		

16	MDB (WH) Dry season	100-150 SL	61	0.0	0.0	54.1	45.9	0.0	100.0	distinguished between filamentous and non-filamentous alga but here pooled, detrital component greatest during periods of low flow
17	MDB (R) Dry season	Range = 34 – 396 TL, majority <80 TL	98	0.0	0.0	33.3	0.0	66.7	100.0	
Average				2.5	1.1	63.0	20.8	9.0	96.5	
SE				0.9	0.6	7.6	6.6	4.2	1.8	

Table 2. Regressions statistics (intercept, slope and F value) for comparisons of isotopic values of sizes classes of *Nematalosa erebi* and putative food sources (see Figure 3). Also given are the lower and upper 95% confidence limits of the slope. n.s. – p>0.05, * - p<0.05 and *** - p<0.001.

Source	Consumer size class	Isotope	n	Intercept	Slope	Lower 95%ile	Upper 95%ile	F value
Benthic algae	<100 mm	$\delta_{13}\text{C}$	64	-11.32	0.39	-0.07	0.84	2.95 _{n.s.}
		$\delta_{15}\text{N}$	63	8.37	0.52	0.27	0.77	18.17***
	>100 mm	$\delta_{13}\text{C}$	73	-22.16	0.22	0.10	0.35	12.71***
		$\delta_{15}\text{N}$	72	7.13	0.44	0.26	0.63	22.91***
Terrestrial vegetation	<100 mm	$\delta_{13}\text{C}$	58	-44.85	-0.55	-0.96	-0.14	7.10*
		$\delta_{15}\text{N}$	58	10.15	0.29	0.03	0.56	4.98*
	>100 mm	$\delta_{13}\text{C}$	55	-30.67	-0.10	-0.48	0.27	0.30 _{n.s.}
		$\delta_{15}\text{N}$	55	7.74	0.53	0.32	0.74	26.80***
Zooplankton	<100 mm	$\delta_{13}\text{C}$	54	-12.44	0.52	0.26	0.77	16.94***
		$\delta_{15}\text{N}$	52	7.54	0.38	0.25	0.52	32.45***
	>100 mm	$\delta_{13}\text{C}$	56	-16.91	0.33	0.11	0.54	9.81***
		$\delta_{15}\text{N}$	54	6.75	0.31	0.19	0.43	28.39***

Table 3. Summary of results of multiple regression analyses for comparisons of isotopic values of sizes classes of *Nematalosa erebi* and putative food sources. n.s. - p>0.05, * - p<0.05, ** - p<0.01 and *** - p<0.001.

Isotope	Size class	Source	Estimated slope	t value	F value
^{13}C	small	Benthic algae	0.024	0.236 _{n.s.}	$F_{3,29} = 167.7^{***}$
		Terrestrial vegetation	0.198	1.163 _{n.s.}	
		Zooplankton	0.699	5.294***	
	large	Benthic algae	0.278	3.257**	$F_{3,31} = 193.8^{***}$
		Terrestrial vegetation	0.463	3.465**	
		Zooplankton	0.218	2.027 _{n.s.}	
^{15}N	small	Benthic algae	0.069	0.322 _{n.s.}	$F_{3,24} = 0.992^{ns}$
		Terrestrial vegetation	-0.276	-1.232 _{n.s.}	
		Zooplankton	0.500	4.229***	
	large	Benthic algae	0.079	0.589 _{n.s.}	$F_{3,25} = 5.967^{**}$
		Terrestrial vegetation	0.296	1.449 _{n.s.}	
		Zooplankton	0.115	1.172 _{n.s.}	

List of Figures

Figure 1. The distribution of *Nematalosa erebi* within freshwater regions of Australia. Unshaded areas do not contain *N. erebi*. Region delineation is based on general similarities in climate and catchment physiography as well as biogeographic variation in fish species distributions (Unmack 2013). The approximate location of studies used here is given and denoted by the number in which studies are listed in Supplementary information.

Figure 2. Frequency distributions of spatial variation in stable isotope values ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) for potential source material (i.e. aquatic and terrestrial primary producers and zooplankton) and for the two size classes of the consumer *Nematalosa erebi*. Sample sizes are 94, 75, 68, 93 and 82 for algae, terrestrial vegetation, zooplankton, large *N. erebi* and small *N. erebi*, respectively.

Figure 3. Carbon and nitrogen isotope values ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in ‰) of muscle or fin tissue of *Nematalosa erebi* plotted against isotope values of potential food sources (algae, terrestrial vegetation and zooplankton). Each point represents the mean value from an individual site within each study. Closed symbols = large fish (>100mm SL) whereas open symbols = small fish (<100mm SL). Unbroken lines represent significant regressions between consumer and producer values for large fish whereas broken lines are for the smaller size class. Sample sizes and regression statistics are given in Table 2.

Fig. 1

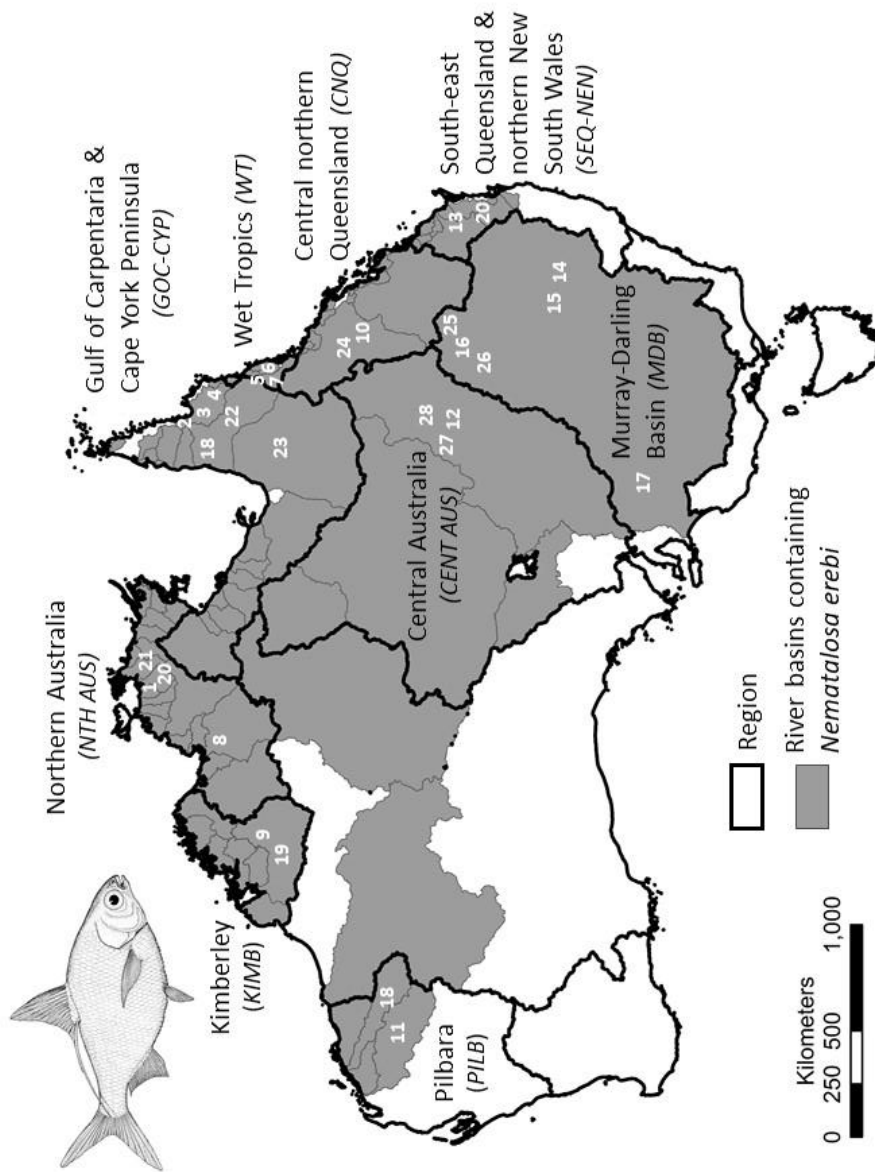


Fig. 2

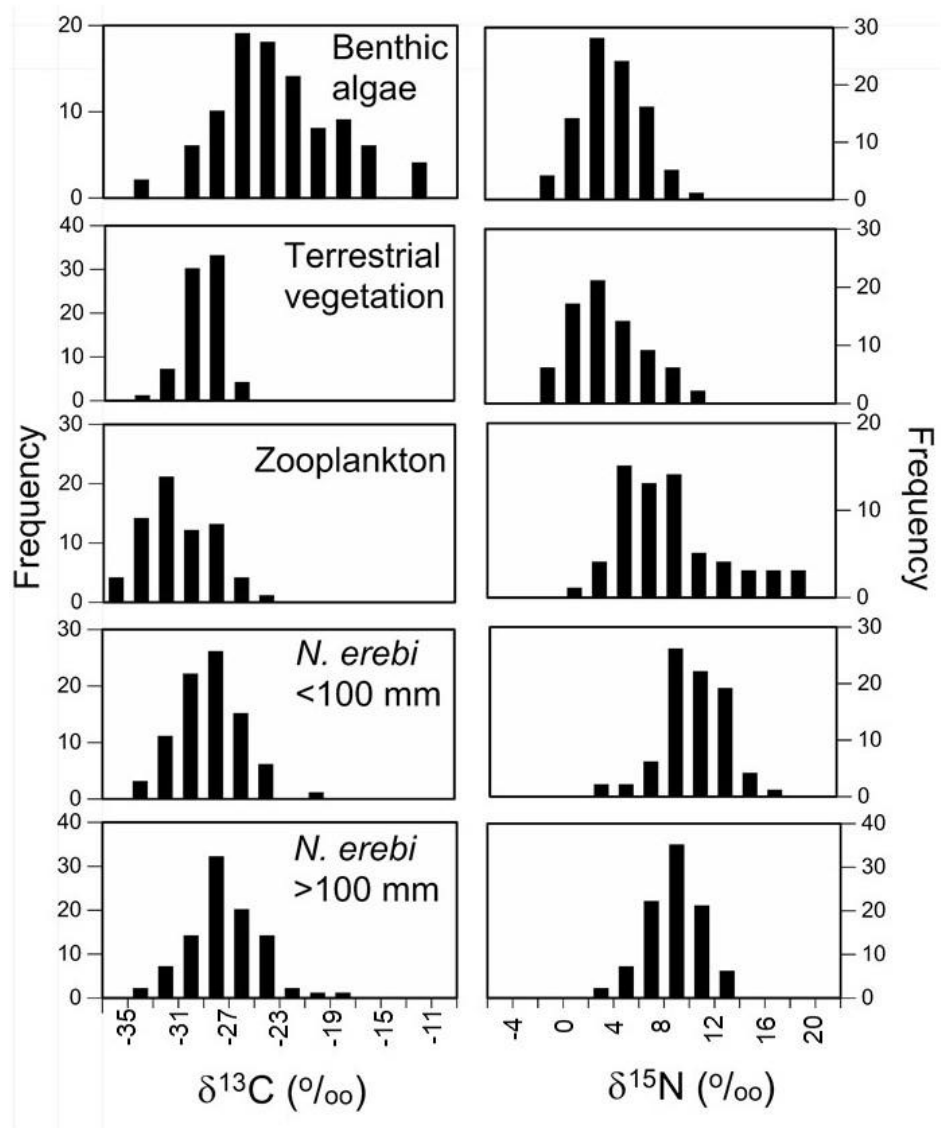


Fig. 3

