



Incorporation of local dissolved organic carbon into floodplain aquatic ecosystems

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Abstract Environmental flow releases in lowland Australian rivers are currently timed to avoid high-carbon production on floodplains. Moreover, return flows (water draining from floodplains back into rivers) are avoided if there exists a risk of introducing deoxygenated “blackwater” into the main channel.

This concern has restricted the range of possible watering scenarios being considered by environmental flow managers. We utilised a series of blackwater flows in the lower Murrumbidgee floodplain, Australia, in 2016 and 2017 to determine the origin and trophic contribution of blackwater dissolved organic carbon (DOC) in a floodplain wetland. We demonstrate a consistent difference in the isotope signature of blackwater DOC compared to both dissolved inorganic carbon (DIC) and river water DOC, explained by the greater contribution of floodplain vegetation (including the river red gum *Eucalyptus camaldulensis*) to blackwater DOC. Stable carbon isotope signatures suggest a contribution of blackwater to algal production, whereby microbial-mediated conversion of blackwater DOC into DIC may create opportunities for primary autotrophic productivity. This carbon signature was incorporated by the common yabby *Cherax destructor*. In the main river channel, *C. destructor*, the native gudgeon *Hypseleotris* spp. and the introduced European carp *Cyprinus carpio* may utilise the same basal carbon source. The use of small to moderate floodplain inundation with return flow to the river, properly monitored, would ameliorate the risk of hypoxia while providing the benefit of floodplain-derived DOC and associated increases to in-stream productivity.

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Introduction

The Flood Pulse Concept, based on large river–floodplain systems, envisions the floodplain as exerting a major influence on primary and secondary production in large lowland rivers (Junk et al. 1989). In these settings, carbon entering the river from floodplains during high flows may be converted by bacteria into forms available to higher-level consumers (Edwards and Meyer 1990), providing an important subsidy to river ecosystems (Baldwin et al. 2016). The contribution of particulate and dissolved carbon can change with flow conditions, with the primary source of carbon in consumers shifting to terrestrial carbon during higher flows (Zeug and Winemiller 2008; Huryn et al. 2001; Reid et al. 2008; Hladysz et al. 2012). In spite of dissolved organic carbon (DOC) being a major form of carbon flux from floodplains to rivers (Thoms 2003; Mitrovic and Baldwin 2016), few studies have traced this carbon source through to consumers, and much of the focus on DOC transport from floodplains in recent studies has concerned potential detrimental impacts on river water quality (Whitworth and Baldwin 2016; Liu et al. 2020).

High levels of dissolved organic carbon (DOC) in waterways yield a characteristic black or brown colour, and these waters are commonly referred to as “blackwater”. When heterotrophic prokaryotes consume DOC, they also draw dissolved oxygen from the water column. If the concentration of DOC is sufficiently high and bioavailable, oxygen may be consumed at a rate greater than can be replenished by diffusion from the atmosphere or water column photosynthesis, resulting in hypoxia (Kerr et al. 2013). However, the concentration of DOC is not always sufficiently high to cause anoxic conditions, nor is it always bioavailable. High DOC is a characteristic of humic or “dystrophic” waterbodies, and where this is a perennial feature low bioavailability of DOC may prevent de-oxygenation (Meyer and Edwards 1990).

Within Australia’s largest river system, the Murray Darling Basin, blackwater conditions lead to hypoxia when DOC concentrations are particularly high and coincident with warm water temperatures (Whitworth et al. 2012). These events typically occur when large floodplains are flooded after long periods of organic carbon accumulation, as often occurs following

drought-breaking rains, particularly where flooding is reduced by water resource development. Rewetting of floodplains provides the conditions for the uptake of DOC from decayed vegetation as a pulse available for consumption by microbes (Langhans and Tockner 2006). Fish kills associated with hypoxic events are common under these circumstances (Gehrke et al. 1993; McKinnon and Shepherd 1997; Baldwin et al. 2001; Townsend and Edwards 2003; Baldwin and Whitworth 2009; King et al. 2012). Similar events have been observed elsewhere, including in the Paraguay River, Brazil (Hamilton et al. 1997) and the Atchafalaya River, Louisiana (Fontenot et al. 2001).

The breaking of the “millennium” drought in south-eastern Australia, after a decade of near record low inflows (2000–2009), resulted in near record high inflows, and several floodplains were simultaneously inundated in the Murrumbidgee and Murray rivers in the spring and summer of 2010/2011 (Whitworth et al. 2012). The event remobilised several hundred thousand tonnes of DOC (Whitworth and Baldwin 2016). Hypoxic blackwater plumes with dissolved oxygen (DO) < 4 mg L⁻¹ extended over 1800 km of river and persisted for up to 6 months in some locations (Whitworth et al. 2012; Kerr et al. 2013). Dissolved oxygen concentrations of at least 2 mg L⁻¹ are required to sustain fish and other large aquatic organisms (King et al. 2012). Murray crayfish (*Euastacus armatus*) were observed emerging from the water in large numbers (King et al. 2012), and extensive fish kills occurred. Events such as these have led to a reticence amongst flow managers to allow flows to return to rivers following floodplain inundation, in spite of the possible subsidy to riverine productivity that may be derived from water high in DOC and nutrients (Wolfenden et al. 2018).

Demonstration of the uptake of blackwater carbon into ecosystems remains a challenge. A comprehensive global review by Roach (2013) found no stable isotope studies that had demonstrated a lateral interaction between river channel and floodplain affecting basal production sources. Several recent Australian studies have monitored the response of river productivity and biota to return flows, though without linking these to stable isotope or other chemical tracers. Cook et al. (2015) reported a doubling of primary productivity in the Murray River after water drained from the Barmah floodplain in

2011. Wolfenden et al. (2018) monitored return flows through the Redbank Weir in the lower Murrumbidgee River during the 2014–2015 environmental watering year. They found a modest increase in river nutrient concentration with a return flow but no evidence of significant ecological responses, though trophic interactions between in-stream consumers and floodplain DOC and terrestrial carbon were not explored.

Dissolved inorganic carbon (DIC) can be sourced from carbonate minerals, atmosphere or groundwater sources and can be incorporated into food-webs via algal production (Ishikawa et al. 2015). Blackwater consists primarily of DOC, which is less easily incorporated directly into food-chains. However, heterotrophic fungi and bacteria may make DOC bio-available through respiration, which may subsequently be transferred to autotrophs (Ishikawa et al. 2015) or directly to grazing fauna (Hagvar et al. 2016), as conceptualised in Fig. 1.

Our aim in this study was to test whether blackwater DOC is an important basal carbon source in floodplain

ecosystems, and whether the influence of carbon derived from DOC can be traced into higher levels of a floodplain aquatic ecosystem using stable isotopes of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$). Our hypotheses were first; that blackwater DOC, being derived from terrestrial floodplain autotrophic sources, would differ in carbon stable isotope composition to DIC, having a more depleted $\delta^{13}\text{C}$ signature reflecting terrestrial vegetation inputs, and may also differ in $\delta^{13}\text{C}$ to river water of lower DOC concentration. Second, we hypothesised that signatures of consumers would reflect the incorporation of DOC-derived C into the food web indicating that DOC-derived C is an important source of C when it is present in high concentrations. In this study, we sampled across a range of floodplain aquatic environments during and following a blackwater event, testing for relationships between the stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotope signatures autotrophic carbon, DOC, DIC surface organic matter and consumers.

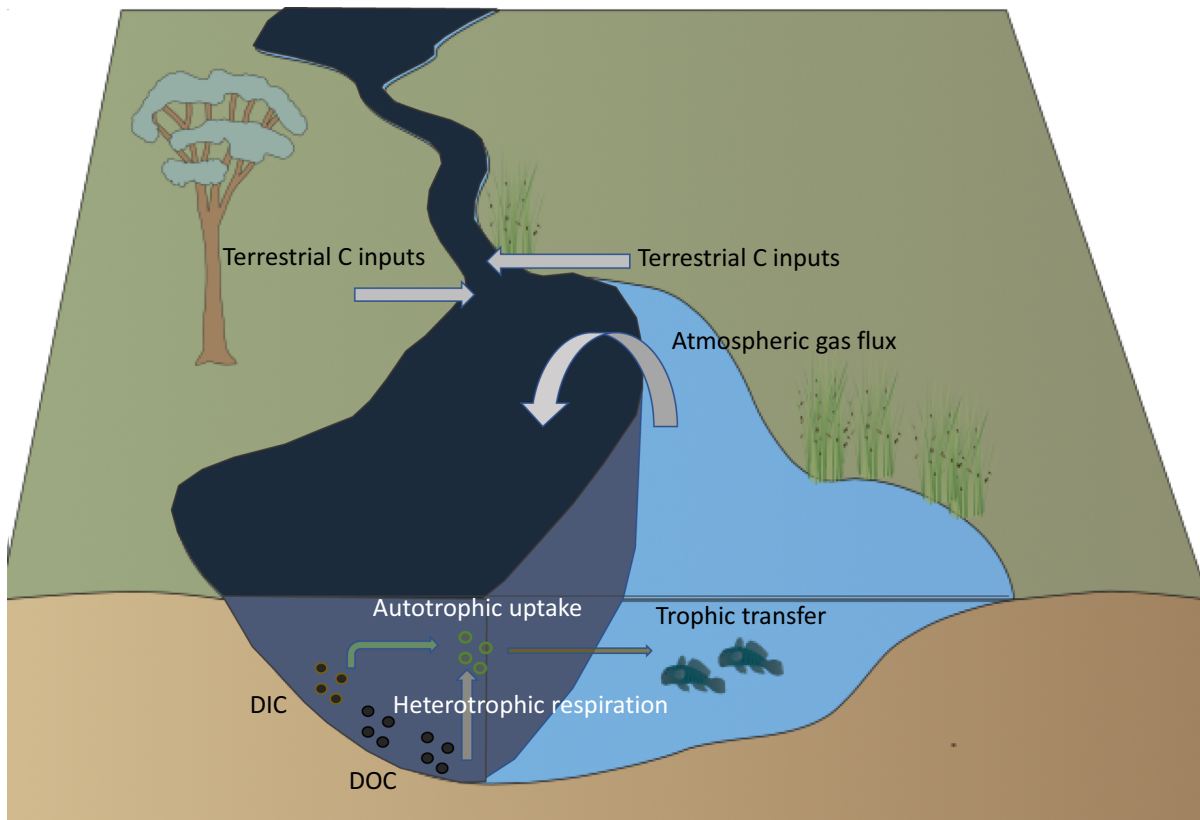


Fig. 1 Conceptual diagram of blackwater carbon uptake into aquatic ecosystems

Methods

Study site

The study was conducted within the Lower Murrumbidgee River and adjoining floodplain areas within Yanga National Park in south-western New South Wales (34°24'S, 143°48'E) (Fig. 2). Mercedes Swamp is a shallow floodplain lake adjacent to the Murrumbidgee River, vegetated by dense growth of the rhizomatous and perennial tall spike-rush (*Eleocharis sphacelata* and *E. acuta*, species utilising the C₃ photosynthetic pathway). Dense rhizomatous growth and production of a thick litter layer by this species largely excludes the growth of C₄ grasses during both inundated and dry phases. The River Red Gum *Eucalyptus camaldulensis* (C₃) is a large tree, which forms a sparse canopy to the margins of Mercedes Swamp. Avenue Swamp is a natural floodplain watercourse through which regulated flows are

conducted to larger floodplain lakes further south in Yanga National Park. The groundcover in Avenue Swamp contains both C₃ (sparse *E. sphacelata* and *E. acuta* among others) and C₄ (water couch *Paspalum distichum*) non-woody vegetation. *E. camaldulensis* (C₃) forms a sparse canopy across most of Avenue Swamp and is a major contributor to leaf litter accumulation.

The dates and locations of elements sampled in our sampling periods are provided as Table 1. A pronounced blackwater event occurred in the lower Murrumbidgee floodplain in October–November 2016. The blackwater was held for several weeks at the Uara Creek regulator seven kilometres from the junction with the Murrumbidgee River and prevented from flowing into the main stem of the Murrumbidgee River. During this period, we sampled water and biota in the Uara Creek site (blackwater) and the main river (non-blackwater) simultaneously. We returned to the site in February 2017 and April 2017 to sample the

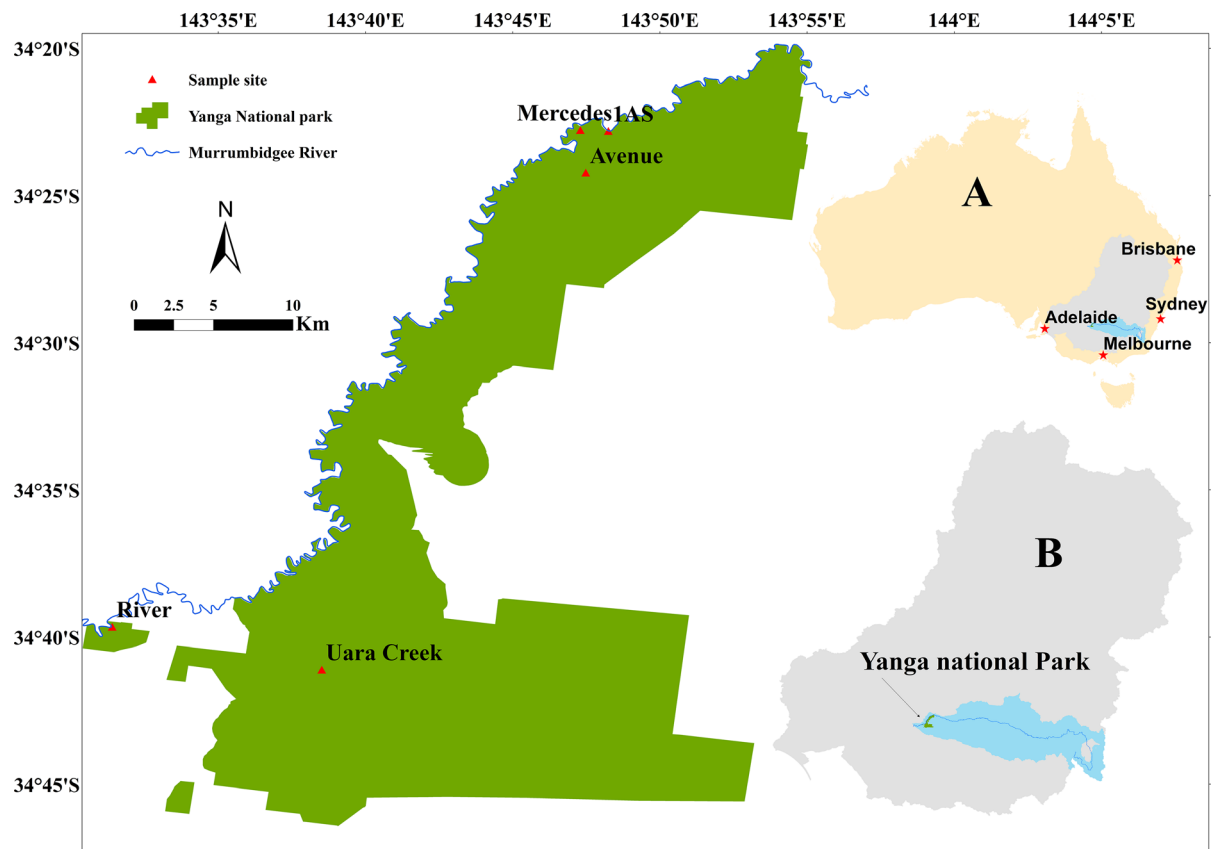


Fig. 2 Lower Murrumbidgee (lowbidgee) floodplain and sampling locations at Yanga National Park, in the context of the Murray Darling Basin (inset a) and the Murrumbidgee River Catchment (inset b)

Table 1 Lowbidgee Floodplain and River sampling for stable isotope analysis and concentration of dissolved organic matter [DOM]

	Feb 2008	April 2008	Nov 2016	Feb. 2017	April 2017
[DOC]	A,M	A,M	U,R	R,U,A,M	R,U,A,M
DIC			U,R	U,R	U,R
DOC			U,R	U,R	U,R
SOM	A,M	A,M	U,R	R,U,A,M	R,U,A,M
<i>Spirogyra</i> spp.	A,M	A,M	U,R		
<i>Cherax destructor</i>	A,M	A	U	R,U,A,M	R,U,A,M
<i>Cyprinus carpio</i>	A,M	A,M		R,A,M	R,A,M
<i>Hypseleotris</i> spp.	A,M	A,M		R,A,M	R,A,M

R = Murrumbidgee River;
 U = Uara Creek;
 M = Mercedes Swamp;
 A = Avenue Swamp

Uara Creek blackwater site 10 weeks and 15 weeks following the blackwater event, in order to test whether blackwater carbon had been incorporated into components of the ecosystem. The blackwater was still present in the Uara Creek over these two sampling events, as evidenced by the relatively high DOC readings (at 40 ppm, nearly an order of magnitude greater than the background river readings, and double that of the nearby wetlands: Fig. 3).

During the 2017 sampling occasions, we also sampled at floodplain locations (Mercedes Swamp and Avenue Swamp) in the northern section of Yanga National Park, as these sites have isotopically distinct floodplain vegetation (Iles et al. 2010). These sites are fed directly by the river through the Redbank Weir and

thus were not influenced by the Uara Creek blackwater event.

We also compared DOC concentration and $\delta^{13}\text{C}$ signatures from collection in 2016/2017 to data collected a decade earlier (Mazumder et al. 2012) following targeted, small environmental flows toward the end of the Millennium drought (February and April 2008) using the same methods. This provided a gradient of blackwater events (i.e. DOC concentrations) over which trophic responses could be assessed (Fig. 4).

Fig. 3 Carbon and nitrogen stable isotope signatures of consumers and sediment and algal sources over time in three floodplain wetlands and the main river channel. See Table S1 for vascular plant values

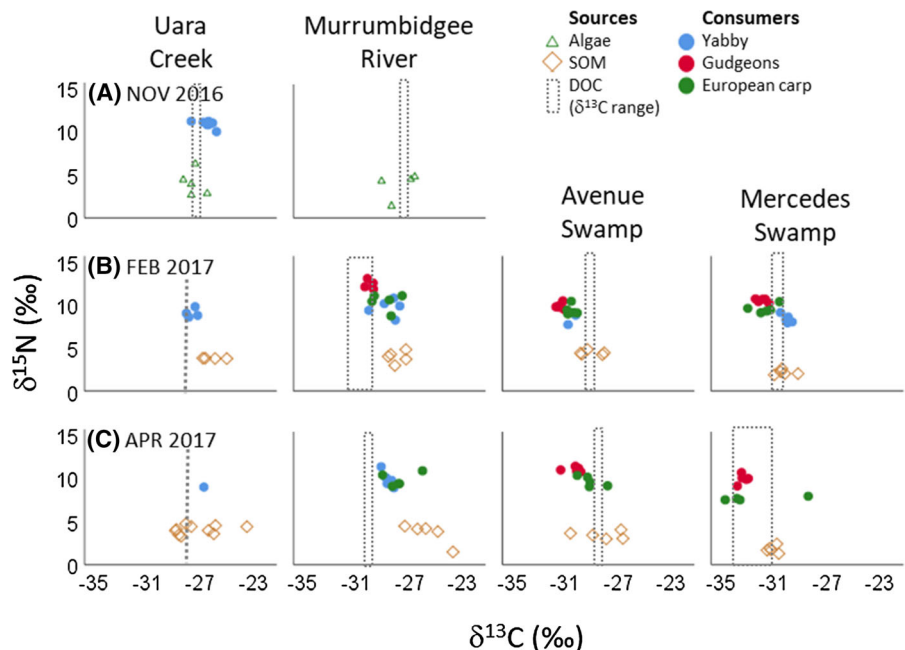
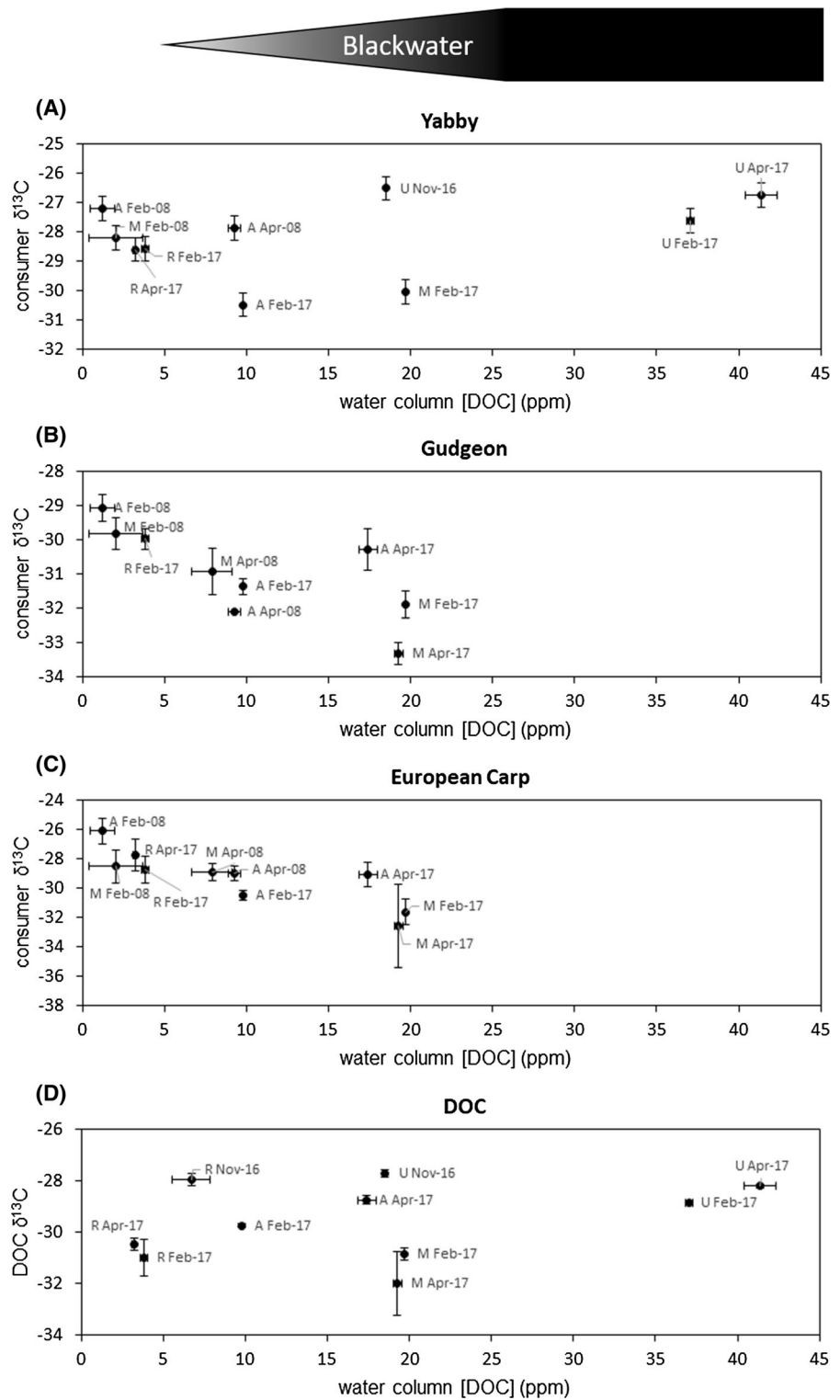


Fig. 4 Comparison of consumer—yabby *C. destructor* (a), Gudgeon (b), European Carp (c)—and DOC (d) $\delta^{13}\text{C}$ signatures across a gradient of blackwater conditions following floodplain environmental flows in December 2007 and natural flooding in late 2016. *U* Uara Creek, *R* Murrumbidgee River, *M* Mercedes Swamp, *A* Avenue Swamp



Sample collection

Water chemistry analysis

Two 30-mL sub-samples of mid-column water were taken using a sterilised syringe (Termo, USA). One subsample was immediately filtered through a glass fibre filter (pore size 0.45 μm ; Sartorius, Germany) for determination of DOC. The other subsample, unfiltered, was used for determination of total nitrogen (TN) and total phosphorus (TP). All these samples were stored at $-20\text{ }^{\circ}\text{C}$ until analysis. Flow injection analysis was used to determine the concentrations of nutrients. TN was measured as $\text{NO}_x\text{-N}$ following autoclave persulfate digestion (Hosomi and Sudo 1986). TP was measured as $\text{PO}_4\text{-P}$ by the ascorbic acid reduction method following autoclave persulfate digestion. A Lachat QuikChem 8000 flow injection analyser (Hach Company, USA) was used for TN and TP analyses. DOC was analysed using a Dohrmann TOC analyser.

Soil and biota sampling

Benthic filamentous algae *Spirogyra* spp. and fresh leaves of the dominant macrophytes *E. acuta* and *E. spachelata* and floodplain tree *Eucalyptus camaldulensis* were collected by hand. Samples of the top 50 mm depth of a mixture of detritus and sediment, representing sediment organic matter (SOM), were collected with a hand corer (60 mm diameter). The importance of SOM is recognised as food source for a range of aquatic species including freshwater fish (Pen et al. 1993). All samples were frozen immediately after collection for transportation and storage until time of analysis. *C. destructor*, *Cyprinus carpio* and *Hypseleotris* spp. were caught using two large fyke nets (12 mm stretched mesh, 1.1 m diameter, 7.5 m wings) and two small fyke nets (2 mm mesh, 0.4 m diameter, 1.2 m wings) at each site facing upstream and downstream directions. These nets were set in the afternoon and retrieved the following morning. *C. destructor*, *C. carpio* and *Hypseleotris* spp. could not be sampled in the main stem during the November 2016 sampling event, due to the high flow velocity.

The muscle tissue from the posterior dorsal of the yabby was used for stable isotope analysis after exoskeleton removal. Fish digestive tracts were

removed prior to muscle dissection and preserved in ethanol for later gut content analysis. Tissue samples were dried at $60\text{ }^{\circ}\text{C}$ for 24 h and ground to a fine powder with a mortar and pestle. Algae samples were cleaned of debris using deionised water followed by drying at $60\text{ }^{\circ}\text{C}$ for 24 h before being ground to a fine powder with a ball mill (Retsch MM-400, Retsch, Haan, Germany). Each core of sediment was sub-sampled for the analysis of carbon (bathed in 0.1 N HCl for 1 h; Bunn et al. 1995) and nitrogen (unbathed) and dried at $60\text{ }^{\circ}\text{C}$ for 24 h and ground to a fine powder.

Stable isotope analysis

Stable isotope analysis seeks to quantify the contribution of dietary sources used by consumers using the ratio of heavier to lighter isotopes, typically of carbon (^{13}C : ^{12}C or $\delta^{13}\text{C}$) and nitrogen (^{15}N : ^{14}N or $\delta^{15}\text{N}$). The carbon stable isotope ratios are particularly useful in identifying the provenance of carbon contributing to an organism's diet, in that fractionation of isotope ratios during assimilation—though variable among consumers—is typically 0–1‰ for each trophic level (Zanden and Rasmussen 2001). Nitrogen isotopes show greater fractionation between trophic levels (typically 3–4‰) and are therefore useful in distinguishing trophic positions and overall food-chain length from autotrophic carbon to highest predator (Zanden and Rasmussen 2001).

The $\delta^{13}\text{C}$ -DIC signatures of waters were analysed by continuous-flow Delta V advantage isotope ratio mass spectrometry (IRMS). Results were reported as per mil (‰) deviation from the international carbonate standard, NBS19 with a precision of $\pm 0.1\text{ }^{\circ}\text{‰}$. The DOC concentration and $\delta^{13}\text{C}$ -DOC were analysed at UC-Davis Stable Isotope Facility using a total organic carbon analyser interfaced to a PDZ Europa20-20 IRMS utilising a GD-100 gas trap interface. Results were reported as per mil (‰) deviation from the NIST standard reference material with an analytical precision of $\pm 0.6\text{ }^{\circ}\text{‰}$.

In the laboratory, the biota samples were oven dried at $\sim 60\text{ }^{\circ}\text{C}$ for 48 h and then ground to a fine powder with a mortar and pestle that had been cleaned and dried between samples to prevent cross contamination. Stable carbon and nitrogen isotope analysis of biota samples ($n = 5$ for each component) from each site

was performed at the Australian Nuclear Science and Technology Organisation (ANSTO) in Sydney, Australia, with a continuous flow isotope ratio mass spectrometer (CF-IRMS), model Delta V Plus (Thermo Scientific Corporation, USA), interfaced with an elemental analyser (Thermo Fisher Flash 2000 HT EA, Thermo Electron Corporation, USA). Results are accurate to ± 0.3 parts per thousand for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. Stable isotope values were reported in delta (δ) units in parts per thousand (‰) relative to the international standard and determined as follows:

$$X(\text{‰}) = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000$$

where $X = \delta^{13}\text{C}$ or $\delta^{15}\text{N}$, and $R = {}^{13}\text{C}/{}^{12}\text{C}$ or ${}^{15}\text{N}/{}^{14}\text{N}$, respectively. The carbon to nitrogen molar ratio (C:N) is closely related to the lipid content of tissue. Lipid content in muscle tissue can affect the $\delta^{13}\text{C}$ values, so normalising $\delta^{13}\text{C}$ values for lipid content is needed for accurate interpretation of food web data (Hoffman et al. 2015). Thus, the lipid content for muscle tissues were normalised using mathematical equations suggested by Post et al. (2007) if the C:N ratio was greater than 3.5: $\delta^{13}\text{C}_{\text{normalised}} = \delta^{13}\text{C}_{\text{untreated}} - 3.32 + 0.99 \times \text{C:N}$.

Statistical methods

Univariate data (DOC concentration; $\delta^{13}\text{C}$ -DOC; $\delta^{13}\text{C}$ -DIC; algae $\delta^{13}\text{C}$) collected in November 2016 were assessed for among site differences (i.e. Ura Creek blackwater versus non-blackwater Murrumbidgee River) with a series of unpaired t-tests. An additional comparison was made between $\delta^{13}\text{C}$ -DOC and algae $\delta^{13}\text{C}$ for each location.

Univariate data collected in February 2017 and April 2017 were assessed for differences among four study locations with one-way Analyses of Variance (ANOVA). Differences in isotope signatures of dominant floodplain C3 plant sources were also assessed with separate one-way ANOVAs for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. Post hoc comparisons were assessed via a Bonferroni correction. Where Levene's tests revealed violation of the homogeneity of variance assumption, outputs were instead reported for Welch's ANOVA, and Games–Howell post hoc comparisons, as these tests are robust to heterogeneous variances. All analyses were

completed using SPSS v 25 (IBM) using a 0.05 significance level.

Results and discussion

DIC, DOC, SOM and probable sources

Macrophyte stable carbon isotope signatures are presented in Table S1. The River Red Gum *E. camaldulensis* was enriched in relation to the two species of *Eleocharis* which form the dominant understory of the forest at Avenue Swamp and also the dominant vegetation at Mercedes Swamp. There was no significant difference in carbon or nitrogen isotope signature between the two species of *Eleocharis*, or between living leaves and leaf litter derived from *E. camaldulensis* (Tables S2 and S3). *E. camaldulensis* living and litter leaf material was heavily depleted in $\delta^{15}\text{N}$ compared to *Eleocharis* spp., except at Avenue Swamp.

Surface organic carbon $\delta^{13}\text{C}$ reflects the dominant vegetation and did not change greatly between sampling times (Fig. 3). At most sites SOM $\delta^{13}\text{C}$ reflects the contribution of carbon from *E. camaldulensis*, which was relatively enriched compared to other carbon sources. Mercedes swamp SOM reflects the more depleted signature of the dominant species, *E. sphacelata* and *E. acuta* and the enriched range associated with *E. camaldulensis* was absent. The Avenue SOM reflects the full range of vegetation $\delta^{13}\text{C}$ at the site, ranging from -31 to -27 ‰.

During the November 2016 sampling phase, DOC concentrations were \sim threefold higher in Ura Creek than in the main river, though there was much less difference in the $\delta^{13}\text{C}$ -DOC of these locations (Table 2; pairwise comparison P values < 0.001 in Table S4). In contrast, $\delta^{13}\text{C}$ -DIC, which was substantially more enriched than $\delta^{13}\text{C}$ -DOC, varied on average by 4.9‰ between blackwater and river water (Table 2). The algae sampled in both locations at this time had stable isotope signatures that were indiscernible from that of the DOC (Table 2) but could not have been derived from DIC.

The $\delta^{13}\text{C}$ signature of DOC in the water column in some locations is consistent with that of SOM (Fig. 3). For example, they were highly aligned at Mercedes Swamp, both reflecting the depleted signature of the *Eleocharis* reedbeds, the dominant macrophyte. The

Table 2 Summary of mean (± 1 standard error) $\delta^{13}\text{C}$ signatures of dissolved inorganic carbon (DIC), dissolved organic carbon (DOC) and algae at the Uara Creek (blackwater) and Murrumbidgee River (non-blackwater) sites in November 2016

	Mean $\delta^{13}\text{C}$ ($\pm 1\text{SE}$)		Mean difference (‰)	Among site comparison	
	Uara Creek (blackwater)	Murrumbidgee River (non-blackwater)		<i>t</i> value	<i>P</i> value
DIC	-15.6 ± 0.1	-10.7 ± 0.1	4.9	26.53	< 0.001
DOC	-27.7 ± 0.1	-28.0 ± 0.1	0.3	2.15	0.06
Algae	-27.5 ± 0.3	-26.0 ± 1.8	1.5	0.84	0.45
DOC versus algae	Uara Creek		0.2	0.73	0.48
	Murrumbidgee River		2.0	1.09	0.34

wetland acts as a hydrological sink with long residence times, and there is little through-flow of DOC from alternative sources. In the Avenue, SOM and DOC $\delta^{13}\text{C}$ signatures reflect the presence of both the dominant River Red Gum *E. camaldulensis* and the *Eleocharis* understory. Uara Creek $\delta^{13}\text{C}$ -DOC was significantly enriched relative to other sites during the blackwater event (pairwise comparison *P* values < 0.001 in Table S5) consistent with the enriched SOM signature at this site (Fig. 3), and strongly aligning to the $\delta^{13}\text{C}$ of *E. camaldulensis*. For this reason, it would appear that the floodplain vegetation is dominating DOC, and algal carbon isotopes reflect this source rather than DIC. In the main channel there is a divergence between SOM and DOC $\delta^{13}\text{C}$ signatures (Fig. 3), with river DOC too depleted in $\delta^{13}\text{C}$ to be reflecting inputs from the *E. camaldulensis* lining the channel and likely to be incorporating algal sources.

Differences in nutrient concentrations between sampling sites (Figure S1), may have some bearing on nitrogen isotope ratios. Phosphorus concentrations at Avenue Swamp were an order of magnitude higher than the other sites. This is possibly due to phosphorus inputs from the active rookery site that supports several thousand Ibis in the *E. camaldulensis* forest, though *E. camaldulensis* leaves also release bioavailable phosphorus on decomposition (Baldwin 1999). If this is the case, it is likely that the total nitrogen (twice the concentration of the river water) is partly influenced by bird faecal inputs, which would explain the unusually elevated $\delta^{15}\text{N}$ of the *E. camaldulensis* leaves at the site (enriched more than 2‰ compared to other sites) (Kendall et al. 2007). This would also

explain the 2‰ enrichment in the $\delta^{15}\text{N}$ of surface organic matter at The Avenue compared to Mercedes Swamp.

Consumer diet in relation to DOC and SOM

Given the $\sim 1\text{‰}$ enrichment fractionation in $\delta^{13}\text{C}$ typical between autotrophic source and consumer (Zanden and Rasmussen 2001), *C. destructor* $\delta^{13}\text{C}$ was in the range of average DOC (= SOM) for Mercedes Swamp, and the Uara Creek blackwater DOC (= Algae) (Fig. 3). Within the River, the enrichment between DOC and *C. destructor* $\delta^{13}\text{C}$ in February (2.4‰) and April (2.2‰), and the more enriched nitrogen isotope ratio suggests two trophic levels between this source and *C. destructor* (Fig. 3). Only two *C. destructor* were captured at The Avenue (both February 2017) and we refrain from drawing conclusions for this site on this basis.

Neither European Carp nor Gudgeon could be sampled in the Uara Creek blackwater site. However, sampling in 2017 was undertaken to assess the diet and trophic position of fish under similar conditions on the floodplain post-flood. That is, DOC concentrations were similar to the Uara Creek blackwater event (~ 20 ppm) at Mercedes Swamp in both February and April 2017, while Avenue Swamp reached this concentration by April 2017. In February 2017, Avenue Swamp had a DOC concentration (~ 10 ppm) between that of the river and blackwater and is therefore useful for purposes of comparison.

In both river and wetland settings the DOC $\delta^{13}\text{C}$ signature is closer to the signatures of the fish

Hypseleotris spp. and *C. carpio* than is SOM and (where measured) DIC. Within the main river in 2016/17, the $\delta^{13}\text{C}$ signature of the two species overlapped. DOC-derived carbon is a possible source, being depleted 1.2‰ in relation to the *Hypseleotris* $\delta^{13}\text{C}$ signature though up to 2.4 ‰ for *C. carpio* (Fig. 3). The $\delta^{13}\text{C}$ signature of fish within the Avenue and Mercedes Swamps was more depleted than in the river channel (Fig. 3), and too depleted to be linked directly to DOC.

Comparing these data to our previous survey (Mazumder et al. 2012), there is a trend of increasingly depleted $\delta^{13}\text{C}$ signatures under higher water-column DOC concentrations for both species on the floodplain (Fig. 5; $r^2 = 0.69$, $P < 0.001$). Site- and time-of-year-specific comparisons reveal multiple instances where there is a coupling of shifts in DOC concentration and consumer $\delta^{13}\text{C}$, thereby suggesting blackwater has a trophic influence. When DOC concentrations were high there were simultaneous large depletions in $\delta^{13}\text{C}$ in all three consumers between February 2008 and February 2017 at Mercedes and Avenue swamps and in both fish species between April 2008 and April 2017 in Mercedes swamp (there were no comparison data for *C. destructor*). The direction of these shifts is equivalent to northern-hemisphere studies which show depletion in $\delta^{13}\text{C}$ signatures in freshwater autotrophs when DIC is not limited (versus enrichment during DIC limitation) (Hollander and McKenzie 1991; Staal et al. 2007). Although we do not have DIC concentration data, or DIC- $\delta^{13}\text{C}$ data for the entirety of our time-series sampling periods, our results emphasise the need for further investigation of the processes (and isotopic fractionations) involved in the transfer of carbon from the dissolved inorganic phase to higher trophic levels.

Conclusions

In the semi-arid lowland rivers of south-eastern Australia, plant litter is thought to be the main source of carbon contributing to blackwater (Hladyz et al. 2011), with leaves contributing most of the DOC (O'Connell et al. 2000). On the lower Murrumbidgee River floodplain in south-western Australia, the blackwater dissolved organic carbon (DOC) signature corresponds to that of the dominant plant of the floodplain, the River Red Gum *E. camaldulensis*,

previously known to be associated with the leaching of high concentrations of DOC (McMaster and Bond 2008). Incubation studies have demonstrated that approximately one-third of leaf material is bioavailable to micro-organisms (Baldwin 1999; Kreutzweiser and Capell 2003). As an important component of the wetland, *Eleocharis* reed-swamp further depletes the isotopic signature of local DOC.

We found a strong association between the $\delta^{13}\text{C}$ signature of algal and that of DOC across locations, but little association with the $\delta^{13}\text{C}$ signature of DIC. The same bacterial respiration that leads to deoxygenation may also be converting DOC into forms available for autotrophic uptake and incorporation into the aquatic food web. Soil surface organic carbon stable isotope signatures also reflected the dominant vegetation. We observed a gradient of sediment organic carbon isotope depletion from the relatively $\delta^{13}\text{C}$ -enriched *E. camaldulensis* that lined the Uara Creek and main river, through the mixed *E. camaldulensis*/*Eleocharis* understory of The Avenue, to the $\delta^{13}\text{C}$ -depleted *Eleocharis* dominance at Mercedes Swamp.

The common Yabby *C. destructor* is enriched in $\delta^{13}\text{C}$ approximately 1.3‰ relative to DOC and algae in the blackwater event (Table 2). This level of fractionation is consistent with the view that algae rather than SOM are the dominant carbon source for this crustacean species in blackwater, and that the $\delta^{13}\text{C}$ of algae is influenced by DOC. The native Gudgeon *Hypseleotris* and the introduced European Carp *C. carpio* were consistently enriched in $\delta^{15}\text{N}$ in the main river compared to the wetlands and in the range expected if DOC were an important basal source. Wetland fish were too depleted in $\delta^{13}\text{C}$ to be accessing carbon from a source utilising DOC. These results suggest that the re-introduction of blackwater DOC to the main river stem is likely to have a strong beneficial effect on in-stream fish.

Within the lower Murrumbidgee River floodplain, high concentrations of DOC in distributor creeks and wetlands is clearly reflected in the isotopic composition of elements of the aquatic ecosystem, including higher trophic-level consumers. This strongly suggests an important role for non-hypoxic blackwater in enhancing the process of riverine productivity. Note that other important macronutrients such as phosphorus may also contribute to the process especially while water is resident on the floodplain (Knowles et al.

2012; Kobayashi et al. 2013). The high concentrations of phosphate in Avenue Swamp wetland appears to be associated with an active rookery, though phosphorus is actively leached from *E. camaldulensis* leaf litter as biologically available orthophosphate (Glazebrook and Robertson 1999; Baldwin 1999). Wolfenden et al. (2018) also found enhanced phosphate-levels in lower Murrumbidgee wetlands (up to 40 times higher than in the river channel). Given that primary production in the Murrumbidgee river is phosphate-limited (Vink et al. 2005), phosphate-rich wetland water might provide an important additional impetus to riverine ecosystem metabolism and productivity (Knowles et al. 2012; Wolfenden et al. 2018) in relation to non-hypoxic blackwater.

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Data availability All original data are available from the corresponding author upon reasonable request.

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