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Carbon Uptake in Surface Water Food Webs Fed by Palaeogroundwater

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Key Points:

- We report the uptake of old carbon in the surface water food webs of a semiarid zone wetland fed by groundwater of the Great Artesian Basin (GAB), Australia
- Stable carbon and nitrogen isotopes were used in conjunction with ^{14}C to determine connection between groundwater and surface water food webs
- We found ^{14}C-depleted carbon from old groundwater contributed to aquatic food webs with very little dilution by modern atmospheric carbon; fish were up to 11 ka and algae 23 ka

Supporting Information:

- Data Set S1

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Abstract

The use of ^{14}C to elucidate sources of carbon within freshwater aquatic ecosystems is challenging the assumption that modern autochthonous carbon dominates energy flows. We measured the uptake of old carbon through several trophic levels of a wetland fed by groundwater of the Great Artesian Basin, Australia, the largest artesian basin in the world. Stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) and radiocarbon (^{14}C) were used to quantify food chain links and connection between groundwater and surface water food webs. Our results suggest that old groundwater was the dominant carbon source even at the highest trophic levels, with predatory fish returning apparent carbon ages of up to 11 ka. Stable isotope analysis ($\delta^{13}C$ and $\delta^{15}N$) identified trophic links between fish, aquatic insects, and algae with smaller contributions from particulate organic matter to the food webs. As natural mound springs and associated wetlands are the only source of reliable water during dry periods over vast areas of the western Great Artesian Basin, the result has potential implications for the interpretation of archaeological artifacts associated with indigenous passage within the interior.

Plain Language Summary

The Great Artesian Basin, the largest and deepest artesian basin in the world, contains water up to millions of years old, and this water discharges to create small wetlands in the dry interior of Australia. We examined the source and transfer of carbon within an ecosystem fed by groundwater from the Great Artesian Basin, using stable and radioactive isotopes. In the first demonstration of groundwater contribution to ecosystem carbon in semiarid or arid regions, we found very old carbon to be the dominant carbon source to all levels of the food chain, including fish, which returned ages of up to 11,000 years old. Our results have implications for the dating of artifacts from interior Australia, where these wetlands were utilized by aboriginal peoples for millennia.

1. Introduction

The use of radiocarbon (^{14}C) to elucidate sources of carbon within freshwater aquatic environments has challenged long-held assumptions that modern autochthonous carbon dominates energy flows (Bellamy & Bauer, 2017). The ^{14}C is a naturally occurring radioisotope tracer that allows the identification of different ages of carbon in food webs, up to a maximum of ~40 ka. Various studies across a range of climatic settings have shown that older sources of particulate organic carbon and dissolved organic carbon (DOC) associated with soil or sediments may be introduced to rivers by various weathering and erosion processes. These include the glacial ice melt and thawing of permafrost in the Arctic (Hood et al., 2015; Singer et al., 2012; Spencer et al., 2014; Vonk et al., 2013) and the erosion of sediments associated with river deposits in temperate and tropical regions (Kip et al., 2010; Marwick et al., 2015; Mayorga et al., 2005; McGuire et al., 2010). The organic carbon (OC) associated with these processes was found to be old (e.g., 21–29 ka; Vonk et al., 2013).

Dissolved inorganic carbon (DIC) may also contribute to aquatic food webs. This can be sourced from carbonate minerals, atmospheric CO_2, or groundwater sources and can be incorporated into algal production (Ishikawa et al., 2015). Ishikawa et al. (2014) found that periphyton commonly carries a distinctly old ^{14}C signature compared to terrestrial vegetation that is subsequently transferred to consumers. Carbon originating from methane up to 50 ka has been shown to contribute to carbon assimilation in freshwater floodplain (DelVecchia et al., 2016) and subterranean estuarine (Brankovits et al., 2017) ecosystems. A growing body of evidence suggests that ^{14}C-depleted carbon or old carbon is contributing to aquatic food webs and to a

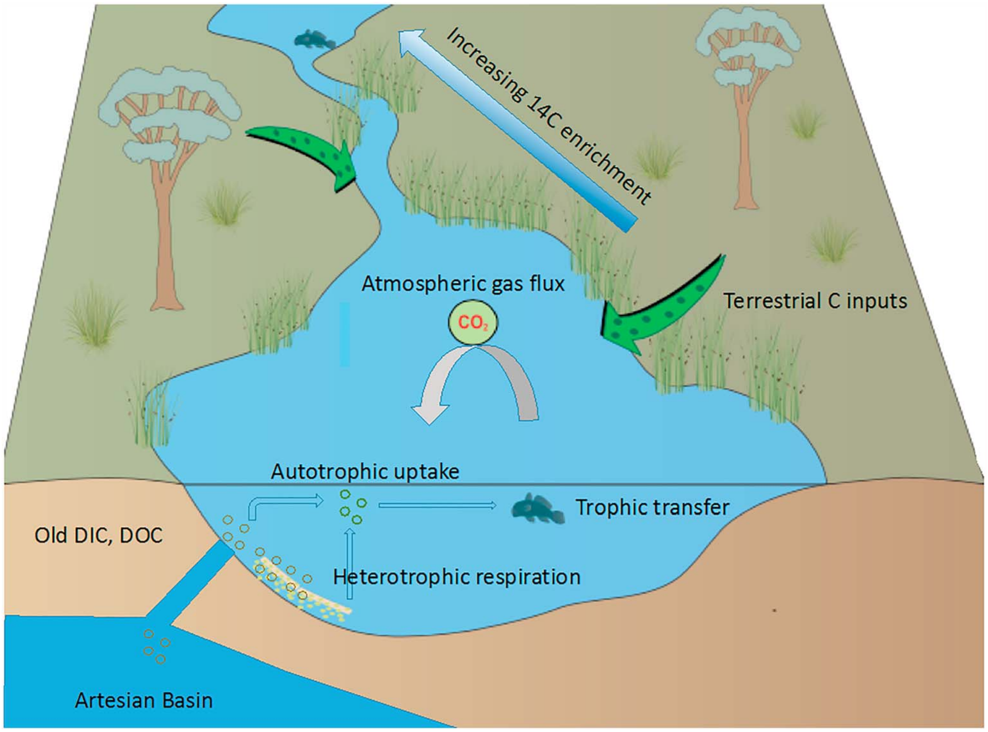

Figure 1. Conceptual model of carbon pathways relevant to the uptake of old carbon for in-stream fauna. Old DOC and DIC (brown circles) are transferred to the wetland, where heterotrophic respiration makes carbon available to autotrophs (green circles), competing with modern terrestrial and atmospheric carbon inputs. DOC = dissolved organic carbon; DIC = dissolved inorganic carbon.

greater extent than previously realized, particularly in environments of low terrestrial productivity (Fellman et al., 2015; McCallister & del Giorgio, 2012).

There are two primary pathways whereby old carbon is incorporated into in-stream food chains (Figure 1). First, heterotrophic fungi and bacteria may make ^{14}C-depleted bioavailable DOC as a result of respiration, which may subsequently be transferred to grazing fauna (Hågvar et al., 2016). Second, autotrophs may incorporate ^{14}C-depleted DIC during photosynthesis but may also utilize dissolved CO_2 derived from heterotrophic respiration of ^{14}C-depleted DOC (Ishikawa et al., 2015). Heterotrophic bacteria utilize DOC and DIC, which may be the dominant source of carbon, particularly in systems receiving sedimentary-sourced OC such as in glacial lakes where it may be several decades before autotrophic algae introduce modern carbon to the waters (Bardgett et al., 2007; Ishikawa et al., 2015). Headwater streams in very old landscapes such as the Kimberly region in Western Australia may also discharge old DOC, where incubation experiments by Fellman et al. (2015) demonstrated this old DOC to be bioavailable. The source of the old DOC was thought to be microbial degradation of ^{14}C-depleted soil carbon, though they also raised the possibility of groundwater autotrophs using ^{14}C-depleted carbon derived from carbonate weathering or heterotrophic respiration of ^{14}C-depleted soil carbon. Fellman et al. (2015) further suggested that old carbon in groundwater may be contributing to downstream food webs, which may make this an important contributor to groundwater-dependent river-floodplain ecosystems, hot spots for productivity in arid and semiarid landscapes.

Several studies have demonstrated that the old carbon (i.e., ^{14}C depleted) mobilized from various geomorphological processes is being incorporated into the lower trophic levels in the aquatic food web. Caraco et al. (2010) found that old carbon material, several millennia in age, provides a subsidy to zooplankton on the Hudson River, New York, USA. Stoneflies in the hyporheic zone of the Nyack Floodplain, Montana, USA, that were dated at over 7 ka (DelVecchia et al., 2016), develop entirely underground in the absence of autotrophic modern carbon sources, with carbon derived from methane. Higher trophic orders are more likely to derive carbon from a range of old and modern sources, evidenced with moderate ages for subarctic fish and predatory invertebrates (Hågvar et al., 2016). Fellman et al. (2015) found that

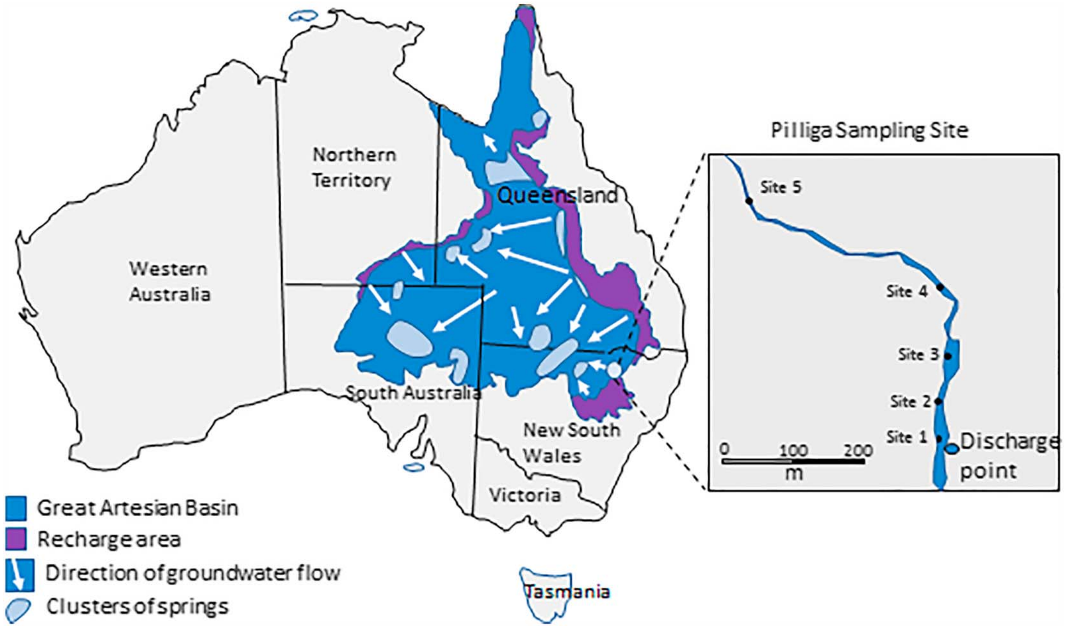

Figure 2. Pilliga sampling sites in relation to the Great Artesian Basin, Australia, after (V. Post, 2012).

OC associated with glacial deposits provided a substantial food source to macroinvertebrates and juvenile salmonids in the upper Herbert River. Hågvar et al. (2016) demonstrated that very old DOC associated with the glacial meltwater ponds is incorporated into aquatic invertebrates. They found that Chironomids and diving beetles ranged from 1 to 3 ka, though fish were modern. However, in another study, Anadromous fish migrating seasonally into surface waters associated with peat deposits containing old OC (8–12 ka) in Arctic Alaska showed seasonal decreases in ^{14}C (Hågvar et al., 2016). These studies from various climates and landscapes show that once the DOC is incorporated into heterotrophic respiration, old OC may enter various aquatic food webs (termed the freshwater reservoir offset). However, the freshwater reservoir effect has not been demonstrated for the world's arid or semiarid regions (Bellamy & Bauer, 2017), where groundwater contributions to groundwater-dependent ecosystems are likely to be a dominant source of carbon for the entire ecosystems.

Our main aim was to test whether old carbon from a palaeogroundwater from the Great Artesian Basin (GAB), Australia, is being incorporated into a groundwater-dependent ecosystem, thereby providing the first demonstration of its incorporation into semiarid landscapes. The GAB is the largest and deepest artesian basin in the world underlying 22% of the Australian continent (Mudd, 2000), some 1.71×10^6 km^2. The age of groundwater in the GAB ranges from thousands of years old in the eastern recharge zones to millions of years in the discharge zones (Mudd, 2000). The predominantly western groundwater flow feeds an extensive network of natural (and subsequently artificial) springs extending through semiarid and arid NSW, Queensland, and South Australia (Figure 2). This study investigates a watercourse fed by GAB groundwater to determine (i) the carbon signatures (^{14}C and ^{13}C) of DIC and DOC and (ii) the quantity and mechanisms whereby old carbon is incorporated into autotrophs, herbivores, and higher-level consumers. Our hypothesis is that, given the low productivity of autotrophs, the absence of surface hydrological input, and the high residence times of groundwater within the basin, old OC will not only dominate autotrophic production but will also be transferred to higher consumers to a degree exceeding observations in other aquatic systems.

2. Methods

Samples were collected from the Pilliga stream, a tributary of the Namoi River 105 km west of Narrabri, NSW, Australia. The Pilliga stream is a ~2-km-long, 30-m-wide shallow waterbody (Figure 2). The main source of water flowing into this stream is from groundwater directly from the GAB. This well is under artesian pressure and was constructed in 1902. Water was collected from the bore discharge point and water;

sediment and biota were collected from five sites (S1 to S5) along the stream in May 2014 (Figure 2). From the discharge point to sites 1–5 were 22, 43, 105, 220, and 328 m.

Surface water samples were obtained using a peristatic pump. An artesian groundwater sample was collected from the wellhead of the Pilliga bore. Water quality variables (electrical conductivity, oxidation-reduction potential, dissolved oxygen [DO], temperature, and pH) were recorded prior to sampling. Water samples were collected from an in-line 0.45-μm (hydrochemistry, $^{14}C_{DIC}$, and alkalinity titrations) and 0.22-μm ($\delta^{13}C_{DOC}$, $^{14}C_{DOC}$, and $\delta^{13}C_{DIC}$) polyethersulphone high-capacity filters depending on the analysis. Total alkalinity values were determined in the field by acid-base titration using a HACH digital titrator. Full details of the methodology for sample collection are provided in Meredith et al. (2012).

Samples of aquatic food web components (biota samples) were collected from each site and included the mosquito fish *Gambusia holbrooki*, the freshwater shrimp *Paratya australiensis*, aquatic insects (Corixidae larvae), filamentous algae, particulate organic matter (POM), and sediment organic matter (SOM). Fish, shrimp, and aquatic insects were collected using a dip net (mesh size: 250 μm). Filamentous algae were collected from the streambed by hand and washed with stream water to minimize debris in the sample. Then it was stored for laboratory analysis. POM was collected onto a 0.8-μm pore size glass filters via an in-line filter unit with a 50-ml sterilized syringe attached to the filter unit. SOM was collected with a hand corer from the top 1 cm of sediment (Melville & Connolly, 2003). All samples were immediately frozen and kept in the dark in a sealed container at −20 °C until laboratory preparation and analysis.

3. Analytical Techniques

3.1. Waters

The $\delta^{13}C_{DIC}$ signatures of waters were analyzed by isotope ratio mass spectrometry (IRMS). Results were reported as per mil (‰) deviation from the international carbonate standard, NBS19 with a precision of ±0.1‰. The DOC concentration and $\delta^{13}C_{DOC}$ were analyzed at UC Davis Stable Isotope Facility using a total OC analyzer interfaced to a PDZ Europa20-20 IRMS utilizing a GD-100 gas trap interface. Results were reported as per mil (‰) deviation from the National Institute of Standards and Technology (NIST) standard reference material with an analytical precision of ±0.6‰. The water samples were also analyzed by ion chromatography for anions and inductively coupled plasma-atomic emission spectrometry for cations (Meredith et al., 2015).

The ^{14}C content of waters ($^{14}C_{DIC}$ and $^{14}C_{DOC}$) was determined by accelerator mass spectrometry at the Australian Nuclear Science and Technology Organisation (ANSTO) in Sydney, Australia, using the STAR 2MV tandem accelerator. Samples were processed according to the methods outlined in Meredith et al. (2012) and Bryan et al. (2017), respectively. Briefly, the total DIC was processed into CO_2 by acidifying the samples and extracting the liberated CO_2 gas. The CO_2 sample was then sealed in a glass tube with CuO, Ag, and Cu wire and heated at 600 °C for 2 hr and then converted into graphite by reducing it with excess hydrogen gas in the presence of an iron catalyst at 600 °C.

The ^{14}C results were reported in percent Modern Carbon (pMC) normalized against the $\delta^{13}C$ of the graphite and as radiocarbon age in years Before Present where “present” is defined as 1950 Common Era. Saturation indices (calcite), concentration of DIC, carbon dioxide [CO_2], carbonate [CO_3^{2-}], and bicarbonate [HCO_3^{-}] were calculated using the WATEQ4F thermodynamic database in the PHREEQC 3.1.7 programme (Parkhurst & Appelo, 1999).

3.2. Biota

In the laboratory, the biota samples were further washed with Mill-Q water, oven dried at ~60 °C for 48 hr, then ground to a fine powder with a mortar and pestle that had been cleaned with ethanol and dried, between samples to prevent cross contamination. Stable carbon and nitrogen isotope analysis of biota samples ($n = 2$–5 for each component) from each site was performed at the 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 analyzer (Thermo Fisher Flash 2000 HT EA, Thermo Electron Corporation, USA). Results are accurate to 1% for both %C and %N and ±0.3 parts per thousand for $\delta^{13}C$ and $\delta^{15}N$. Stable isotope values were reported in delta (δ) units in parts per thousand (‰) relative to the international standard and determined as follows:

Table 1
Isotopic and Water Quality Data for Pilliga Waters

	DOC	$\delta^{13}C_{DOC}$	DIC	$\delta^{13}C_{DIC}$	$\delta^{13}C_{POM}$	$^{14}C_{DIC}$	$^{14}C_{DOC}$	T	EC	DO	pH
ID	ppm	‰	mmol/L	‰	‰	pMC	pMC	°C	μS/cm	mg/L	
Pilliga Bore	0.3	−22.7	10.0	−7.2		0.4 (±0.08)		36.1	1365	0.9	8.3
S1	0.3	−23.3	10.9	−7.1	−26.2	0.5 (±0.02)	5.1 (±0.06)	35.6	1357	2.7	8.3
S2	0.4	−23.3	9.9	−6.4	−25.0	1.0 (±0.04)		28.9	1221	8.1	8.9
S3	0.5	−22.9	11.3	−6.0	−26.2	2.8 (±0.04)		21.8	1054	7.2	8.7
S4	0.7	−23.5	11.7	−6.5	−27.1	4.5 (±0.05)	21.5 (±0.33)	20.2	1018	6.1	8.6
S5	1.0	−24.2	10.2	−6.2	−24.4	6.3 (±0.07)		17.2	960	8.4	8.7

Note. DOC = dissolved organic carbon; DIC = dissolved inorganic carbon; POM = particulate organic matter; EC = electrical conductivity; DO = dissolved oxygen. (± represents standard error).

$$X(‰) = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1\right) \times 1,000 \tag{1}$$

where $X = \delta^{13}C$ or $\delta^{15}N$ and $R = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$, 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 $\delta^{13}C$ values, so normalizing $\delta^{13}C$ values for lipid content is needed for accurate interpretation of food web data (Hoffman et al., 2015). Thus, the lipid content for muscle tissues was normalized using mathematical equations suggested by D. Post et al. (2007) if the C:N ratio was greater than 3.5: $\delta^{13}C_{\text{normalized}} = \delta^{13}C_{\text{untreated}}$—$3.32 + 0.99 \times$ C:N.

Furthermore, dried and ground algae and fish samples from each site (n = 1–2; Table 1) were sealed in a silica tube with CuO, Ag, and Cu wire and heated to 900 °C for overnight. The resulting CO_2 was converted to graphite using the H_2/Fe reduction method (Hua et al., 2001) in preparation for the AMS analysis. As with the water samples, these were analyzed at the ANSTO in Sydney, Australia, using the STAR 2MV tandem accelerator. The ^{14}C results were reported as pMC normalized against the $\delta^{13}C$ of the graphite and as radiocarbon age in years Before Present.

4. Data Analysis

A source mixing model within a Bayesian framework (SIMMR; Parnell, 2016) was applied to stable isotope data to examine the relative contributions of potential food sources to the diet of *Gambusia*. Mean isotopic ($\delta^{13}C$ and $\delta^{15}N$) values, concentrations (%C and %N) of consumers and diet sources, and enrichment factors were used as inputs to the mixing model to estimate the possible contributions of five potential diet sources (SOM, POM, algae, aquatic insects, and shrimp) to the isotopic makeup of the consumer *Gambusia*.

The fractional contribution of old groundwater carbon (F) to algae and fish was calculated using the following mixing equation (equation (2))

$$F = \left(C_{[\text{fish or algae}]} - C_{[\text{old groundwater}]}\right) / \left(C_{[\text{modern}]} - C_{[\text{old groundwater}]}\right) \times 100 \tag{2}$$

where $C_{[\text{old groundwater}]}$ is 0.4 pMC and $C_{[\text{modern}]}$ is 100 pMC.

5. Results

Similarity between groundwater and surface water samples in minor and major ions suggests that the waters have not undergone major hydrochemical processes after exposure to atmospheric oxygen (Table 2). Na and HCO_3 are the dominant ions in groundwater and surface water, which is consistent with GAB groundwater chemistry (Herczeg et al., 1991). Temperature and DO change with exposure to surface conditions, with temperature decreasing from 36.1 °C in the groundwater to 17.2 °C at S5 (located at the far end of the surface water body; Figure 2), and DO also increases from 0.9 mg/L in groundwater to 8.4 mg/L at S5. This influx of DO and decrease in temperature do not change the pH of waters but do cause a decrease in EC.

DOC concentrations increased from 0.3 ppm in the groundwater (and at S1) to 1 ppm at S5. This corresponds with a depletion in $\delta^{13}C_{DOC}$ values from groundwater (−22.7‰) to S5 (−24.2‰) at the end of the flow path, suggesting an influx of DOC slightly more depleted in $\delta^{13}C$ into surface water body along the flow path. The

Table 2
Hydrochemical Data for Pilliga Waters

	Distance	Ca	Fe	K	Mg	Na	Si	Cl^-	SO_4^{2-}	NO_3^-
ID	m	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
Pilliga Bore	0	3.4	0.04	3.5	0.61	289	8.6	57	0.02	0.07
S1	22	3.4	0.04	3.5	0.60	269	8.5	58	0.06	<0.01
S2	43	3.2	0.03	3.5	0.61	267	8.4	58	0.04	0.03
S3	105	3.6	0.01	3.5	0.69	301	8.1	59	0.04	0.10
S4	220	3.4	0.01	3.5	0.70	291	8.0	59	0.03	0.22
S5	328	3.3	0.01	3.4	0.74	272	7.4	60	0.02	0.07

$\delta^{13}C_{POM}$ is generally not related to the $\delta^{13}C_{DOC}$ of the surface water. For example, at S1 the $\delta^{13}C_{POM}$ is 3‰ lower than the $\delta^{13}C_{DOC}$ at S5. However, at S5 the $\delta^{13}C_{POM}$ and $\delta^{13}C_{DOC}$ are similar (Table 2). This suggests that the source of DOC introduced along the flow path could be from POM. The $^{14}C_{DOC}$ at S1 is very low (5 pMC) and increases to 21.5 pMC at S4. This further suggests that the DOC influx into the surface water is from a modern source.

DIC concentration is uniform between sites suggesting groundwater to be the dominant source. However, the carbon stable isotope signature of groundwater (−7.2‰) is slightly enriched compared to surface waters ($\delta^{13}C_{DIC}$ −6.2‰ at S5). This may indicate the introduction of atmospheric CO_2 gas along the surface water system, also reflected in the increase in $^{14}C_{DIC}$ from a low of 0.4 pMC in the groundwater to 6.3 pMC at S5 (Table 1). The surface water at S5 is still very low in $^{14}C_{DIC}$ suggesting limited dilution by at the time of sampling by atmospheric CO_2, rainfall, or overland flow.

Consistent enrichment in $\delta^{15}N$ away from the discharge point (Figure 3) for all species sampled represents a gradient of incorporation of other sources of N and also suggests highly localized movement of all species including fish and crustaceans. This may be explained by the strong thermal gradient between sites: the limited tolerance of aquatic biota to rapid changes in temperature in these systems (Allan & Craig, 2014) may constrain lateral movement within the stream.

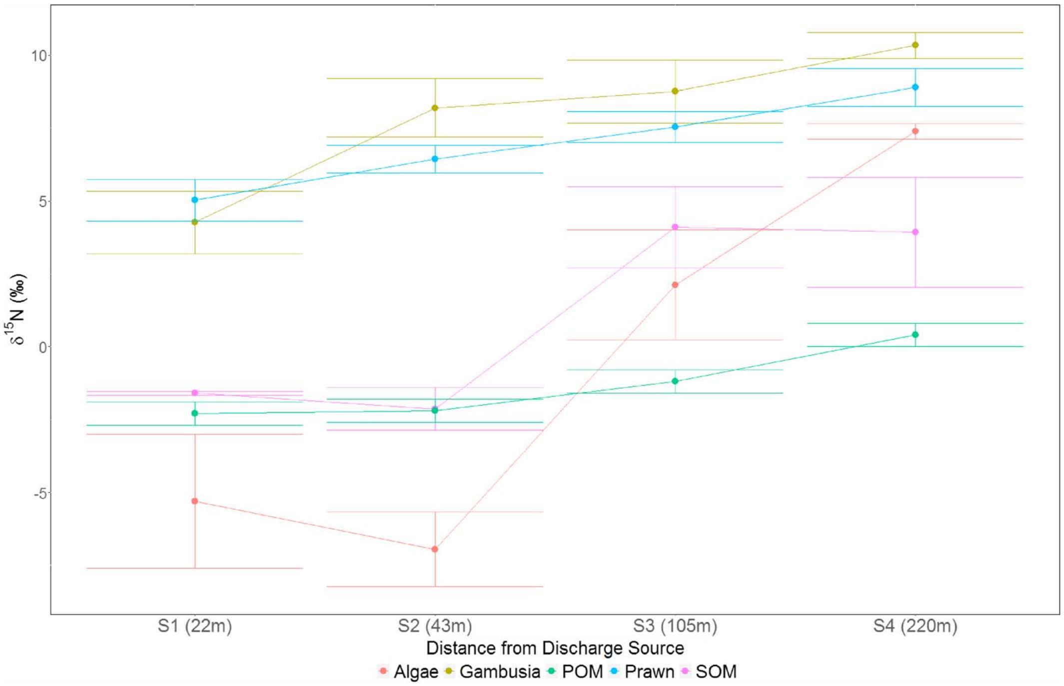

Figure 3. Values of $\delta^{15}N$ along the transect (S1–S4) with distance from the discharge site. (n = 2–5 for each component at each site). POM = particulate organic matter; SOM = sediment organic matter.

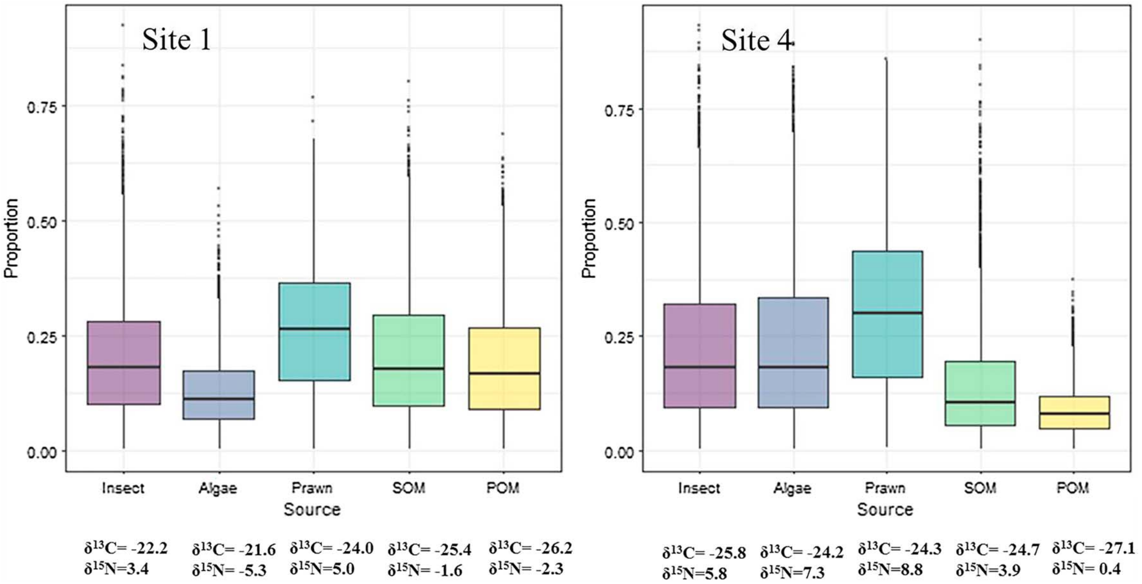

Figure 4. Modeled contributions to the diet of *Gambusia* (mean and 95% confidence range). POM = particulate organic matter; SOM = sediment organic matter.

Because of this evidence of site fidelity, we analyzed dietary contributions to fish at the site level. Mixing model suggests *Gambusia* to be omnivorous at the site, with SOM and filamentous algae both contributing 14% to 24% and the proportional contribution of prawn increasing and aquatic insects decreasing between sites 1 and 4. POM contributions to the diet of *Gambusia* also vary between sites 1 and 4, higher contribution, 12% in site 1 than 10% in site 4 (Figure 4).

The highly depleted δ^{14}C value for *Gambusia* at S1 and a more enriched value in S4 (Table 3) further suggest that fish are using the OC from highly localized sources. Algae δ^{14}C showed enrichment at points along the transect, though was again highly depleted at the furthest point. Overall, the fractional contribution of old groundwater carbon using our source mixing equation was approximately 99.58% in the water column, 82% in algae, and 52% in fish.

6. Discussion

While previous studies have demonstrated the incorporation of old carbon from glacier meltwaters into Chironomids and insect predators (Hågvar et al., 2016), ours is the first study to show the transfer of old groundwater-sourced carbon to high (vertebrate) trophic levels in a semiarid zone climate. The age of the DOC contained in the groundwater in this study (~24 ka) is within the range of the oldest DOC ever recorded (Vonk et al., 2013), and our apparent age of 11 ka for the fish is the oldest for a living vertebrate of which we are aware.

Table 3
The δ^{14}C Values and Apparent Radiocarbon Age of Ecosystem Components From the Four Surface Water Sites

Distance from source (m)	DIC (n = 1)		DOC (n = 1)		Benthic algae (n = 2)		*Gambusia* (n = 2)	
	δ^{14}C	Age (ka BP)	δ^{14}C	Age (ka BP)	δ^{14}C	Age (ka BP)	δ^{14}C	Age (ka BP)
22 (S1)	−994.74	42.07	−949.79	23.97	−941.3	22.71	−753.6	11.19
					−941.76	22.76	−639.9	8.14
43 (S2)	−989.78	36.73			−724.5	10.30		
					−413.4	4.23		
105 (S3)	−971.92	28.64			−796.2	12.72		
					−840.0	14.66		
220 (S4)	−955.64	24.96	−786.46	12.34	−945.7	23.34	−399.2	4.03
					−941.8	22.78	−298.1	2.78

The DIC contributed from the groundwater is ^{14}C depleted, and instream DIC remains low to the furthest sampling point over 200 m from the source. Algal ^{14}C reflects this trend more than DOC signatures, which becomes more enriched. However, heterotrophic respiration of old DOC either in-stream or within the hyporheic zone is likely to be an important pathway by which ^{14}C-depleted carbon is incorporated into the aquatic ecosystem and better explains the enrichment observed in higher organisms. There has been a changing paradigm recognizing the bioavailability of old DOC, previously assumed to be recalcitrant (by virtue of its age). Studies have suggested that old DOC may in some circumstances be more labile than contemporary DOC (Caraco et al., 2010; Vonk et al., 2013).

Our results demonstrate that old groundwater carbon can be the dominant source of carbon for heterotrophic and autotrophic production within associated wetlands. It also shows that the old carbon is taken up into aquatic food web consumers with varying though largely minor levels of dilution by modern atmospheric carbon. The persistence of old carbon along our transect is consistent with findings in glacial meltwater ponds, which may still show old carbon incorporated in ponds that have been subjected to 80 years of atmospheric CO_2 exposure (Hågvar et al., 2016). The potential for strong terrestrial C inputs such as overland flow was not observed when the stream ecosystem was sampled, as evidenced by consistent hydrochemistry. The variability of algal ^{14}C was greater than that of $^{14}C_{DIC}$ and may reflect variation in depth or turbulence influencing exposure to modern atmospheric carbon, though algae were still highly depleted at the end of the sampling reach: algae located at 300 m from the groundwater discharge point yielded a ^{14}C age of 23 ka. The distinct δ^{15}N signatures of fish sampled at various distances from the groundwater source suggest that fish are not moving laterally along the creek, perhaps being inhibited by the strong thermal gradient. Fish at the furthest sampling point had a ^{14}C age of 4 ka, which is enriched compared to highly depleted signatures closer to the spring (11 ka) but still easily identified as deriving carbon primarily from old sources. The experimental design presented here has potential application in exploring the ecological effects of coal seam gas operations common to the region, where the discharge of old carbon groundwater (e.g., the "produced water" resulting from fracking) can be traced and how it influences ecosystem function identified.

The outcome of this research also has far-reaching implications for radiocarbon dating methods of artifacts associated with groundwater-dependent ecosystems associated with the GAB. Fish bones and mollusk shells represent an important component of hearth and midden remains used for the documentation and dating of aboriginal occupation in the region during the Pleistocene (Long et al., 2014). Not only could fish bone artifacts return errors up to 11 ka but humans and other predators utilizing aquatic biota may also incorporate the old carbon leading to inaccuracies in dating methods that are difficult to constrain in most studies. This is of potential significance when dating artifacts associated with mound springs in the discharge regions of the GAB in South Australia. The natural springs of which there are approximately 600 (Habermehl, 1980) are concentrated around the southwestern margin of the basin where groundwater residence times date too millions of years. Surface flows range from 0.1 to 14 ml/day, commonly producing wetlands and small channels. In arid and semiarid environments of extreme rainfall variability these wetlands may be the primary and only permanent source of water for groundwater-dependent ecosystems. Mound springs have been crucially important to Aboriginal inhabitants of the Australian inland, as evidence by the concentration of artifacts and their significance in oral history (Boyd, 1990; Hercus & Sutton, 1985).

Finally, some consideration needs to be given to groundwater-derived carbon as a potential source of carbon not only to aquatic ecosystems but also to atmospheric carbon flux. Old carbon is respired by bacteria and as a consequence lost to the atmosphere. This source may not be sufficiently accounted for in carbon flux models as old carbon is assumed to be permanently stored. McCallister and del Giorgio (2012) assume a latitudinal gradient in the age of carbon processed by aquatic organisms, with warming in higher latitudes contributing to the "permafrost carbon feedback" (Dean et al., 2018) suggesting that midlatitude contributions may also be stronger than previously thought.

7. Conclusions

To our knowledge, the radiocarbon dates for living vertebrates within this system are the oldest ever recorded at 11 ka. The strength of the old carbon signal through the wetland ecosystem suggests that radiocarbon could be used to trace groundwater influences in surface water ecosystems, with applications including the fate of water produced by groundwater extraction and mining. In the context of the arid interior of

Australia, springs and wetlands fed by the GAB have provided staging posts in the movements of nomadic peoples for millennia, and we emphasize the need for caution in the dating of associated artifacts.

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