

# Carbon and nitrogen stable isotope analysis indicates freshwater shrimp *Paratya australiensis* Kemp, 1917 (Atyidae) assimilate cyanobacterial accumulations

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**Abstract** Large areas of uncompacted cyanobacterial accumulations (or “gyttja”) have been observed in Myall Lake, New South Wales, Australia. To determine whether the cyanobacterial accumulations were assimilated into the local food web, carbon and nitrogen stable isotopes were used to identify the primary food sources of a primary consumer in Myall Lake, the freshwater atyid shrimp *Paratya australiensis*. Suspended particulate organic matter (POM) and the macrophyte *Myriophyllum salsugineum* were identified as major dietary sources of *P. australiensis*. Enriched stable isotope signatures ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) of shrimp from gyttja-affected sites, relative to shrimp from unaffected locations, also indicated that *P. australiensis* were deriving a considerable portion

of their dietary carbon and nitrogen requirements from gyttja. Stable isotope mixing models estimated that cyanobacterial accumulations might constitute up to 69% of *P. australiensis* biomass carbon and nitrogen requirements at gyttja-affected locations. To our knowledge, this is the first study to use stable isotope analysis to trace the assimilation of potentially toxic cyanobacterial accumulations into the trophic pathways of an affected system.

**Keywords** Carbon · Nitrogen · Stable isotope ·  $\delta^{13}\text{C}$  ·  $\delta^{15}\text{N}$  · Cyanobacterial mats · Gyttja · *Paratya australiensis* · Myall Lakes

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Guest editors: J. Wilson, L. Bowling & J. Tibby  
The Myall Lakes: patterns and processes in an unusual coastal lake system in eastern Australia

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## Introduction

Benthic cyanobacterial accumulations are a worldwide phenomenon, occurring in a diverse range of aquatic habitats and a variety of physical conditions (Bauld, 1986). Cyanobacterial accumulations, or mats, have the potential to spread over large areas within affected waterways (Bowling & Baker, 1996) and receive considerable attention from authorities in the form of water quality monitoring, clean-up programmes and public safety assessments (Dasey et al., 2004).

The Myall Lakes is a large coastal lake system on the lower north coast of New South Wales, Australia. The natural and conservational value of the Myall

Lakes system is internationally recognised, with the area being listed as a Ramsar Wetland of International Importance (NPWS, 2002). In 1999, studies of the limnology and ecology of the Myall Lakes revealed extensive areas (800 ha) of uncompacted, cyanobacterial 'ooze', or gyttja, in the northern-most lake, Myall Lake (Dasey et al., 2004). Taxonomic examination of the gyttja indicated it was largely composed of cyanobacteria (*Aphanothece*) from the order Chroococcales. Toxicity assays of the cyanobacteria comprising the ooze indicated a potential for the production of hepatotoxins (Dasey et al., 2004).

Cyanobacterial blooms are used as a food source by a range of crustaceans including cladocerans (Boon et al., 1994; Matveev et al., 1994), copepods (DeMott & Moxter, 1991; Koski et al., 1999), mysids (Engström et al., 2001), prawns and crayfish (Vasconcelos et al., 2001; Chen & Xie, 2005; Kankaanpää et al., 2005) and crabs (Magalhães et al., 2003; Pinho et al., 2003). Direct and indirect effects of toxic cyanobacterial assimilation into the trophic pathways include the removal of functional taxa crucial to system health, declines in important food sources and prey items and the bioaccumulation of harmful toxins in secondary and tertiary predators. The aim of this study was to determine if the carbon (C) and nitrogen (N) of cyanobacterial accumulations within Myall Lake were being assimilated into the local food webs by primary consumers. To test this, multiple stable isotope tracing was used to identify the major sources of organic matter supporting a key primary consumer in Myall Lake, the freshwater atyid shrimp *Paratya australiensis* Kemp, 1917.

Atyid shrimp form a major component of the overall macroinvertebrate biomass in many aquatic systems (Sheldon & Walker, 1998; Burns & Walker, 2000) and constitute an important link in estuarine and freshwater food webs, comprising a major component in the diet of secondary consumers such as other macroinvertebrates, fish and birds (Miller, 1979; Bunn & Boon, 1993; Leberer & Nelson, 2001; Howell et al., 2004). *Paratya australiensis* is the most widespread freshwater shrimp in eastern Australia, ranging from central Queensland, through south eastern Australia and Tasmania (Williams, 1977; Richardson et al., 2004). It occurs in a wide variety of permanent inland water systems including coastal lakes and estuaries (Davie, 2002), streams (Sheldon & Walker, 1998) and rivers (Hancock &

Bunn, 1997). *P. australiensis* uses a wide range of feeding strategies, ranging from filter-feeding on suspended particulates (Gemmell, 1978); browsing on detritus, algae and other small invertebrates (Bunn & Boon, 1993); and grazing upon microalgae, such as biofilms and cyanobacterial complexes (Burns & Walker, 2000). Given these characteristics, *P. australiensis* appears to be an ideal test organism to assess whether organic matter derived from cyanobacterial accumulations is being assimilated into the food web of the Myall Lake system.

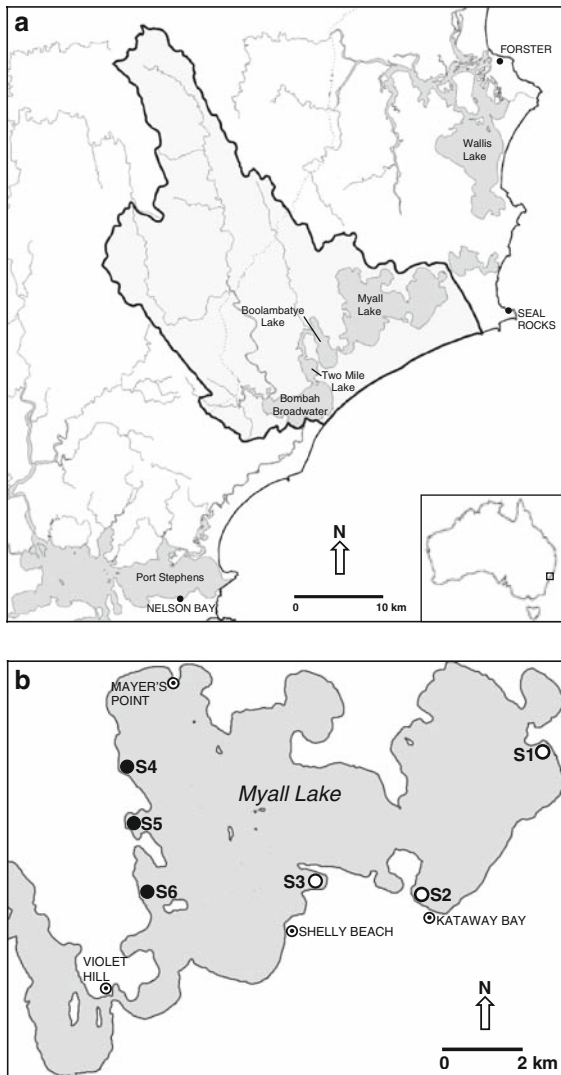
The stable isotopes of C and N are most commonly used to indicate the passage of organic food sources through food webs, with the ratio of  $^{13}\text{C}:^{12}\text{C}$  (or  $\delta^{13}\text{C}$ ) and  $^{15}\text{N}:^{14}\text{N}$  (or  $\delta^{15}\text{N}$ ) in a consumers tissue theoretically matching the C and N ratios of their major food source(s). Stable isotope analysis was conducted on populations of *P. australiensis* from sites containing cyanobacterial accumulations, and control sites containing no gyttja, and compared with the isotopic values of potential major food sources within Myall Lake.

## Materials and methods

### Study area and sampling locations

The Myall Lakes are a coastal lake system comprising three interconnected water bodies situated on the lower north coast of New South Wales, Australia (Fig. 1). The total waterway area of the system is  $>100\text{ km}^2$ , with a catchment area of  $780\text{ km}^2$ . Myall Lake and Bombah Broadwater are the two largest lakes in the system and are connected by Boolambatye Lake. Myall Lake is mostly freshwater with little estuarine vegetation while the Bombah Broadwater, Two Mile and Boolambatye Lakes are slightly saline (dependant upon rainfall). The lower Myall River is a 25 km stretch of waterway that connects the Myall Lakes system with Port Stephens and Tasman Sea to the south.

All sampling sites for this study were situated within Myall Lake. Three control sites (S1–S3) were chosen at Neranie, Kataway Bay and north of Shelly Beach (Fig. 1), based on the absence of any visual cyanobacterial accumulations (gyttja). Three locations containing gyttja (S4–S6) were identified and sampled on the western shore of the lake between Violet Hill and Mayers Point (Fig. 1).



**Fig. 1** Map of (a) the Myall Lakes system and catchment area, and (b) Myall Lake, indicating control sites (S1–S3) and gytija-affected sites (S4–S6)

Physico-chemical properties of the study locations were measured in April 2002 and 2003 using a Yeo-Kal 611 water quality analyser (Yeo-Kal, NSW, Australia). Water temperature was generally uniform across all sampling sites:  $\sim 25^{\circ}\text{C}$  in 2002 and  $20.5^{\circ}\text{C}$  in 2003. Water was only slightly brackish with salinity ranging from  $\sim 2.8$  in east (S1) to 3.3 in west (S6). Chlorophyll *a* (chl *a*) samples taken at all sampling sites in 2003 indicated that the level of chlorophyll *a* in the water column was the same at control and gytija sites ( $\sim 1.5 \text{ mg m}^{-3}$ ), with the exception of control site S2 which had significantly higher chl *a* than all other sites

(SNK post hoc comparisons,  $P < 0.001$ ). This suggests that there was little resuspension of cyanobacterial particles into the water column at sites containing gytija during the sampling period.

#### Sample collection and processing

To determine the sources of carbon and nitrogen used by *P. australiensis*, adult shrimp were collected from all sites in late summer, April 2002 and 2003 and their potential food sources were collected from available sites in April 2003. *P. australiensis* were caught using a 30 cm dip net with a 1 mm mesh. The net was repeatedly swept along the substratum and among stands of reeds in 10–20 cm of water until sufficient numbers of shrimp were caught. *P. australiensis* were kept alive and placed in individual buckets containing field water from their respective sampling sites that had been filtered using a  $0.2 \mu\text{m}$  filter. Shrimp were kept like this for 24 h to ensure their guts were cleared of any traces of food that may influence later stable isotope readings. Following gut clearance, the decapods were frozen and transported to the laboratory. After selecting six shrimp of similar size from each site, their exoskeleton was carefully removed using a scalpel and a muscle sample was obtained and rinsed using distilled water.

Samples of gytija were collected from the three sites within Myall Lake in April 2002 and 2003. Replicate samples of gytija were collected from two areas within each site. All samples were taken from just below the surface of the gytija. Following collection, samples were mixed well to ensure homogeneity and a 100 ml sub-sample was taken and frozen for later analysis.

Water samples for suspended particulate organic matter (POM) analysis were collected in 1 l jars at each site at the time of decapod sampling in April 2003. POM samples were filtered onto pre-combusted Advantec glass fibre filter papers (GC-50; 47 mm  $\phi$ ) under low vacuum and frozen.

Benthic detritus was extracted from hand cores of the topmost 10 cm of sediment in April 2003. This sampling was only conducted at control sites, since the presence of gytija all the way up to the shoreline prevented the collection of uncontaminated sediments at gytija-affected sites. Samples were dried and sieved through a 2 mm and  $250 \mu\text{m}$  mesh to collect the desired size fractions.

In April 2003, samples of rushes (*Baumea juncea*), reeds (*P. australis*), macrophytes (*Myriophyllum sal-sugineum*), charophytes (*Najas marina*) and leaf litter were collected at each site, when present, for stable isotope analysis. For rushes and reeds the leaf-blade sheath was separated from the leaf shoot for separate analysis using a scalpel blade. In the case of *B. juncea* the exposed root sheath was also removed for analysis. Leaf litter was separated into its major components—these primarily being *Melaleuca quinquenervia* and *Casuarina glauca* leaves. All vegetation samples were rinsed with distilled water to remove dirt and detritus and frozen.

All samples for stable isotope analysis were freeze dried to a constant weight using a Dynavac FD3 freeze drier unit. Shrimp, gyttja, plant and detritus samples were weighed and homogenised into a fine powder and transferred into tin capsules for isotope analysis. For POM and biofilm samples, filtered matter was scraped from the surface of filter papers using forceps and sealed in tin capsules for isotope analysis. Analysis of carbon and nitrogen stable isotope ratios and C and N content was carried out using an elemental analyser (Eurovector EA 3000) and mass spectrometer (Isoprime). Ratios of  $^{13}\text{C}:^{12}\text{C}$  and  $^{15}\text{N}:^{14}\text{N}$  are expressed as the relative per mil (‰) between the sample and the conventional international standards (atmospheric nitrogen for  $\delta^{15}\text{N}$  and Pee Dee Belemnite for  $\delta^{13}\text{C}$ )

$$\delta X = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1,000$$

where  $X = ^{13}\text{C}$  or  $^{15}\text{N}$  and  $R = ^{13}\text{C}:^{12}\text{C}$  or  $^{15}\text{N}:^{14}\text{N}$ . Measurements were to a precision of approximately 0.2‰ for  $^{13}\text{C}:^{12}\text{C}$  and  $^{15}\text{N}:^{14}\text{N}$ .

#### Potential food sources

Only *P. australiensis* from the 2003 sampling period were used to identify potential food sources, since this was the only sampling period when all the possible dietary components were also collected. Since fractionation values of C and N isotopes from diet to *P. australiensis* are unknown, broad-range values based upon existing literature were used. *P. australiensis* were predicted to have a  $\delta^{13}\text{C}$  of 1‰ depleted or 2‰ enriched and  $\delta^{15}\text{N}$  1–5‰ enriched relative to potential food sources (Bunn & Boon, 1993; France & Peters, 1997). Any primary sources outside these ranges were considered to be unlikely (or minor) contributors to overall shrimp diet.

Carbon–nitrogen ratios (C:N) were used to characterise the nutritional quality of potential food sources. This technique has been successfully used to investigate the diets and quality of food sources for aquatic snails (Sheldon & Walker, 1997) and decapod crustaceans (Burns & Walker, 2000). The suggested maximum C:N for sustainable invertebrate nutrition is  $\leq 17$  (Russell-Hunter, 1970), with lower C:N values generally equating to increased nutritional quality.

A mixing model was used to assess the feasible individual contributions of the range of potential food sources to the signature of the consumers. Separate models were computed for control and gyttja location shrimp, with gyttja included only in the models of exposed locations and benthic detritus present only in the control models. We used the IsoSource model developed by Phillips & Gregg (2003) for all calculations. This method considers all possible combinations of each primary source contribution (between 0 and 100%) at small increments (in this case 1%). Results are presented as the distribution of feasible solutions per primary source (in the form of histograms), as recommended by Phillips & Gregg (2003). The 1–99 percentile range of source contributions is presented (along with median values), rather than the full range which is sensitive to small values of observations at the limits of the distribution (Phillips & Gregg, 2003). Prior to IsoSource analysis, *P. australiensis* C and N isotopic signatures were adjusted to account for consumer enrichment. Given that C and N trophic enrichment values for *P. australiensis* are unknown, we tested a range of possible values in the model, based upon findings from previous freshwater SIA studies. Trophic enrichment values tested in the mixing model were (for C and N, respectively): 0‰ and 2.2‰ (Johannsson et al., 2001); 1.2‰ and 2.7‰ (Goedkoop et al., 2006); 1.3‰ and 2.2‰ (McCutchan Jr et al., 2003); 1.5‰ and 2.7‰ (Keough et al., 1996); 2‰ and 5‰ (Bunn & Boon, 1993).

#### Data analysis

The  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values for *P. australiensis* were analysed to determine differences among sites. Samples collected in 2002 and 2003 were analysed separately, using a two-factor analysis of variance (ANOVA), with the first factor of Habitat (i.e. gyttja-affected or control) being fixed and orthogonal and

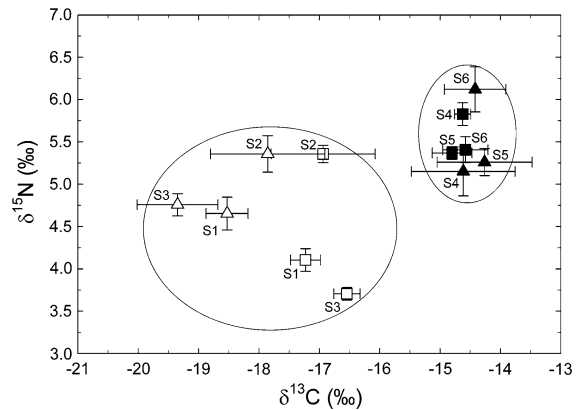
the second factor of Site being random and nested within Habitat. A two-factor ANOVA was used to compare gytija sampled in 2002 and 2003, with the first factor of Year (2002 and 2003) being fixed and orthogonal and the second factor of Site random. Two-factor ANOVAs were also used to compare POM and chl *a* samples, with the first factor of Habitat being fixed and orthogonal and the second factor of Site being random and nested within Habitat. Since samples of the remaining primary sources were not collected from all habitats and/or sites, orthogonal, fixed one-factor ANOVAs were used to test for significant differences between collection sites. No significant differences in any of the primary sources (including gytija) were found between sites; therefore, mean C and N isotopic values were calculated for each primary source and used for comparisons.

## Results

### *P. australiensis* stable isotope signatures

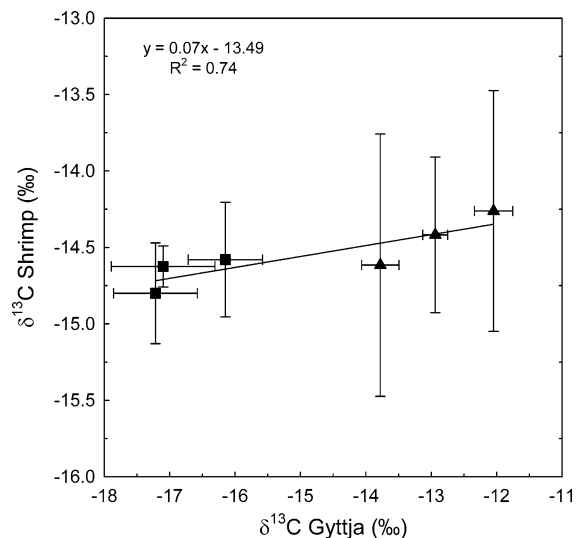
In 2002, the average  $\delta^{13}\text{C}$  stable isotope value of *P. australiensis* sampled from gytija-affected sites was 2.2‰ higher than that of shrimp from control sites ( $-14.7 \pm 0.2$  and  $-16.9 \pm 0.3\text{‰}$ , respectively), representing a significant enrichment in the  $\delta^{13}\text{C}$  value of shrimp exposed to gytija relative to shrimp from control habitats ( $P < 0.001$ ; Fig. 2). In 2003 this trend was stronger still, with the mean  $\delta^{13}\text{C}$  values for *P. australiensis* from gytija-affected sites enriched in  $^{13}\text{C}$  by 4.2‰ over those from control sites ( $-14.4 \pm 0.4$  and  $-18.6 \pm 0.4\text{‰}$ , respectively), again representing a significant enrichment in  $^{13}\text{C}$  ( $P < 0.001$ ; Fig. 2). There was a significant increase in shrimp  $\delta^{13}\text{C}$  with increasing gytija  $\delta^{13}\text{C}$  across sites and times, further supporting gytija as a dietary source (shrimp  $\delta^{13}\text{C} = 0.07$  gytija  $\delta^{13}\text{C} - 13.49$ ,  $P < 0.05$ ,  $R^2 = 0.74$ ,  $n = 6$ ; Fig. 3). The relationship model indicates a shrimp C trophic enrichment factor of 0.07‰ over gytija; however, the accuracy of this value is questionable given the relatively high variability in  $\delta^{13}\text{C}$  for 2003 shrimp samples (Fig. 3).

In contrast to the strong patterns observed for  $\delta^{13}\text{C}$ , the delineation between control and gytija sites was less distinct when comparing  $\delta^{15}\text{N}$  values of *P. australiensis*. In 2002, the average  $\delta^{15}\text{N}$  value of



**Fig. 2** Comparison of carbon and nitrogen isotope values for *Paratya australiensis* collected from sampling sites in Myall Lakes in 2002 and 2003 (squares = 2002 samples; triangles = 2003 samples; open symbols = control sites; black symbols = gytija-affected sites; S1 – S6 = sampling sites). Values represent means  $\pm$  SE ( $n = 6$ )

gytija-exposed shrimp was enriched relative to control shrimp by 1.1‰ ( $5.5 \pm 0.1$  and  $4.4 \pm 0.2\text{‰}$ , respectively); however, this enrichment was not significant, due largely to the significant variation in shrimp  $\delta^{15}\text{N}$  values observed among sites ( $P < 0.001$ ; Fig. 2). In 2003 any trend towards enrichment was weaker still, with gytija site shrimp enriched in  $^{15}\text{N}$  relative to control site shrimp by an average of only 0.6‰ ( $5.5 \pm 0.2$  and  $4.9 \pm 0.1\text{‰}$ ,



**Fig. 3** Relationship between the  $\delta^{13}\text{C}$  of cyanobacterial accumulations (gytija) and the  $\delta^{13}\text{C}$  of *Paratya australiensis* tissue (squares = 2002 samples; triangles = 2003 samples). Points represent mean isotopic values  $\pm$  SE ( $n = 6$ )

**Table 1** Stable isotope signatures, C and N contents and C:N ratios for primary sources from sampling sites in Myall Lake in 2003

Food source	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	C (%)	N (%)	C:N Ratio
POM (24)	$-19.9 \pm 0.3$	$0.1 \pm 0.2$	–	–	$9.2 \pm 0.1$
Benthic detritus (12)	$-27.3 \pm 0.6$	$0.1 \pm 0.5$	$30.1 \pm 3.8$	$0.8 \pm 0.1$	$44 \pm 6.4$
Cyanobacterial accumulation					
2002 sampling (11)	$-16.8 \pm 0.3$	$1.5 \pm 0.1$	$26.2 \pm 0.7$	$3.5 \pm 0.1$	$7.6 \pm 0.3$
2003 sampling (18)	$-12.9 \pm 0.2$	$1.7 \pm 0.1$	$20.6 \pm 0.5$	$2.7 \pm 0.1$	$7.7 \pm 0.1$
<i>Najas marina</i> (6)	$-10.1 \pm 1.1$	$-11.2 \pm 1.7$	$29.0 \pm 0.5$	$3.7 \pm 0.1$	$7.9 \pm 0.1$
<i>Myriophyllum salsugineum</i> (6)	$-15.8 \pm 0.7$	$1.4 \pm 0.4$	$33.8 \pm 1.2$	$2.3 \pm 0.4$	$15.9 \pm 2.3$
<i>Baume juncea</i>					
Leaf blade (12)	$-27.2 \pm 0.3$	$2.6 \pm 0.1$	$37.4 \pm 0.2$	$0.6 \pm 0.1$	$65.0 \pm 5.3$
Leaf sheath (12)	$-27.2 \pm 0.4$	$2.1 \pm 0.2$	$37.8 \pm 0.5$	$0.6 \pm 0.1$	$71.8 \pm 7.6$
Root sheath (3)	$-26.3 \pm 0.2$	$1.5 \pm 0.4$	$37.8 \pm 0.4$	$0.3 \pm 0.0$	$124.6 \pm 18.0$
Combined (27)	$-27.1 \pm 0.2$	$2.2 \pm 0.1$	$37.7 \pm 0.2$	$0.6 \pm 0.0$	$74.6 \pm 5.6$
<i>Phragmites australis</i>					
Leaf blade (3)	$-29.2 \pm 0.1$	$2.8 \pm 0.1$	$38.1 \pm 0.5$	$0.6 \pm 0.1$	$66.1 \pm 11.4$
Leaf sheath (3)	$-29.1 \pm 0.4$	$0.9 \pm 0.3$	$39.1 \pm 0.2$	$0.4 \pm 0.1$	$109.5 \pm 16.0$
Combined (6)	$-29.2 \pm 0.2$	$1.8 \pm 0.4$	$38.6 \pm 0.3$	$0.5 \pm 0.1$	$87.8 \pm 13.1$
<i>Melaleuca quinquenervia</i> leaf litter (12)	$-31.0 \pm 0.2$	$1.6 \pm 0.2$	$44.8 \pm 0.5$	$0.7 \pm 0.1$	$65.8 \pm 6.3$
<i>Casuarina glauca</i> leaf litter (3)	$-30.4 \pm 0.3$	$-1.8 \pm 0.3$	$40.5 \pm 0.3$	$1.8 \pm 0.1$	$23.3 \pm 1.5$

Values indicate mean  $\pm$  SE. Values in parentheses indicate number of samples

respectively). Once again, differences between gyttja and control habitats were overshadowed by significant variation observed among individual sites ( $P = 0.006$ ; Fig. 2). No relationship was observed between changes in gyttja  $\delta^{15}\text{N}$  and the  $\delta^{15}\text{N}$  of shrimp tissues ( $R^2 = 0.17$ ,  $P = 0.40$ ). It is interesting to note, however, that in both years, the  $\delta^{15}\text{N}$  values for *P. australiensis* from control site 2 were more similar to the values of gyttja site shrimp rather than those from the remaining control sites 1 and 3 (Fig. 2).

#### Composition of primary sources

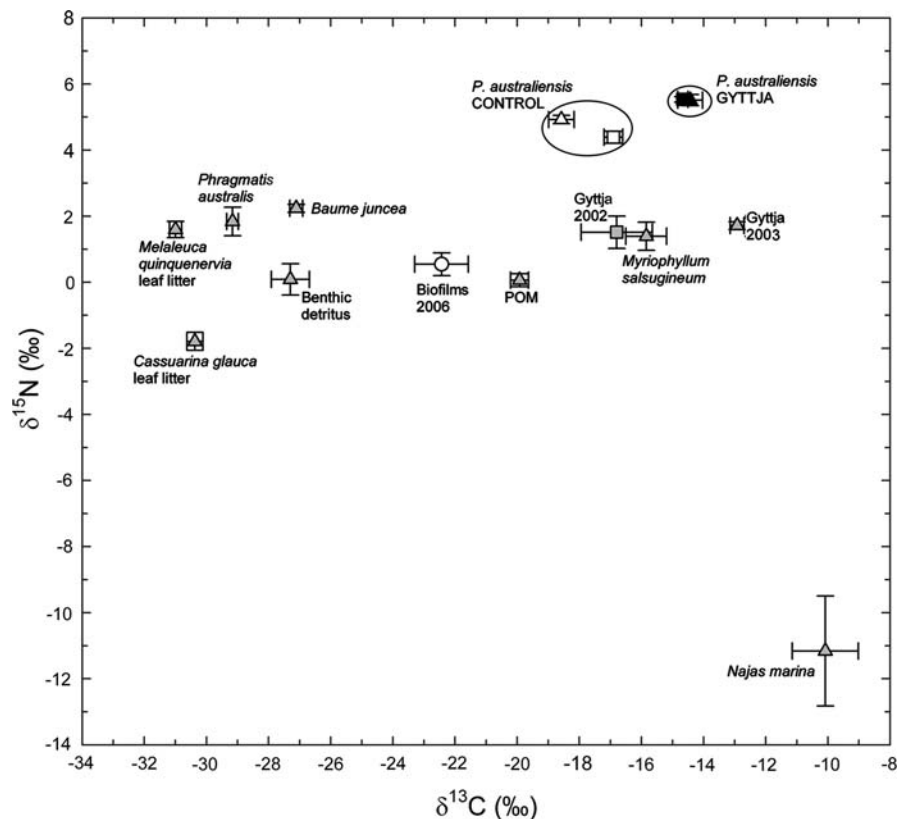
##### Carbon

Among the eight possible food sources identified at both control and gyttja sites, the leaf litter of *M. quinquenervia* and *C. glauca* were most depleted in  $^{13}\text{C}$  ( $-31.0 \pm 0.2$  and  $-30.4 \pm 0.3\text{‰}$ , respectively; Table 1; Fig. 4), while the 2003 cyanobacterial accumulations and the charophyte *N. marina* were the most enriched ( $-12.9 \pm 0.2$  and  $-10.1 \pm 1.1\text{‰}$ , respectively; Table 1; Fig. 4).

Suspended POM ( $-19.9 \pm 0.3\text{‰}$ ) values were depleted relative to the control and gyttja site shrimp by 1.3 and 5.5‰, respectively, and, in combination with other enriched sources, appear as potential dietary sources for *P. australiensis* (Fig. 4). Gyttja sampled in 2002 had significantly depleted  $\delta^{13}\text{C}$  values relative to samples taken in 2003 ( $P < 0.001$ ; Table 1; Fig. 4). While 2002 gyttja values were depleted relative to gyttja site shrimp values, the enriched nature of 2003 cyanobacterial accumulations over *P. australiensis* from affected sites ( $-12.9 \pm 0.2\text{‰}$ ; Table 1; Fig. 4) suggests that this is a substantial source of organic carbon for shrimp at these sites, driving their overall enrichment relative to shrimp from control sites (Fig. 4). The macrophyte *M. salsugineum* appeared enriched (2.8‰) and depleted (1.4‰) relative to control and gyttja site shrimp, respectively (Table 1; Fig. 4) and is also a likely source of organic carbon for *P. australiensis* from all sites.

All other potential carbon sources appeared too depleted in  $^{13}\text{C}$  to be anything other than minor contributors to *P. australiensis* diet (Table 1, Fig. 4). The  $\delta^{13}\text{C}$  values of the individual components

**Fig. 4** Comparison of mean carbon and nitrogen values for *Paratya australiensis*, gytija and primary sources collected from control and gytija-affected sites in Myall Lakes in 2002 and 2003. (squares = 2002 samples; triangles = 2003 samples; open symbols = *P. australiensis* from control sites; black symbols = *P. australiensis* from gytija-affected sites; grey symbols = primary sources). Biofilm values from sampling in 2006 have been included for comparison (open circle). Values represent means  $\pm$  SE



(blades, sheath and roots) of *Baume juncea* rushes and *Phragmites australis* reeds differed very little (0.5‰ and 0.1‰, respectively) and were therefore combined to achieve a whole plant value ( $-27.1 \pm 0.2‰$  for *B. juncea*;  $-29.2 \pm 0.2‰$  for *P. australis*; Table 1; Fig. 4). Benthic detritus was substantially  $^{13}\text{C}$ -depleted relative to suspended POM ( $-27.3 \pm 0.6‰$ ; Table 1; Fig. 4), most likely due to it being largely comprising a combination of reed, rush and leaf litter detritus.

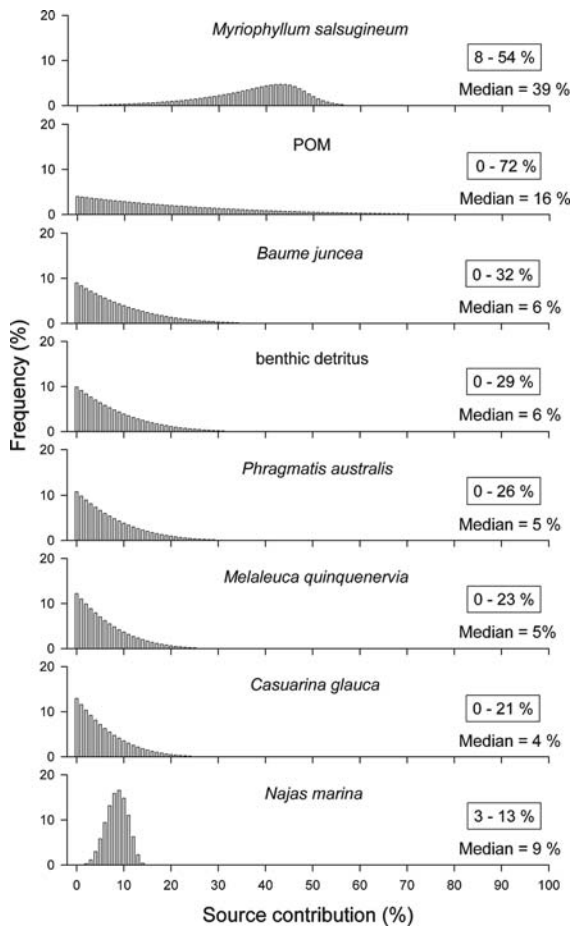
## Nitrogen

Most of the identified primary sources of organic matter were generally  $\leq 5‰$   $\delta^{15}\text{N}$  of control and gytija site shrimp, indicating they are all possibly being used by *P. australiensis* (Table 1; Fig. 5). *Casuarina glauca* leaf litter was considerably depleted in  $^{15}\text{N}$  ( $-1.3$  to  $-2.3‰$ ; Table 1; Fig. 4) relative to shrimp  $\delta^{15}\text{N}$ , suggesting any contribution it might make to *P. australiensis* diet would be minor. Similarly, the extremely negative  $\delta^{15}\text{N}$  of *N. marina* ( $-11.2 \pm 1.7‰$ ; Table 1; Fig. 4) suggests it is not used.

The  $\delta^{15}\text{N}$  values of cyanobacterial accumulations sampled in 2002 and 2003 did not differ significantly ( $1.5 \pm 0.1$  and  $1.7 \pm 0.1‰$ , respectively; Table 1; Fig. 4), which was in contrast to observations made for  $\delta^{13}\text{C}$ . Compared to the other major shrimp food sources identified by  $\delta^{13}\text{C}$  values (POM and *M. salsugineum*), gytija was only slightly enriched over POM (1.5‰), and therefore cannot be solely attributed to the enrichment observed in gytija site shrimp relative to those from control locations (Table 1; Fig. 4).

## C:N ratios

The C:N ratios of primary sources ranged from  $124 \pm 18.0$  for *Baume juncea* root sheaths, to  $7.6 \pm 0.3$  for gytija sampled in 2002 (Table 1). The only primary sources with C:N ratios within the bounds of sustainable invertebrate nutrition ( $\leq 17$ ) were the same sources identified by  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values as being the major possible shrimp food sources - namely suspended POM, gytija and *M. salsugineum* (Table 1). *Najas marina* also had a



**Fig. 5** Feasible contributions of primary food sources to the isotope signatures of *Paratya australiensis* from control locations. Corrections for trophic enrichment have been made for  $^{13}\text{C}$  ( $-2\text{‰}$ ) and  $^{15}\text{N}$  ( $-5\text{‰}$ ). Values in boxes represent 1–99 percentile range of source contributions. Median (50 percentile) for each source contribution is also presented

low C:N ratio ( $7.9 \pm 0.1$ ), indicating a high nutritional potential.

#### Mixing models

Of the range of C and N trophic enrichment values used in the mixing model calculations, only 2‰ C and 5‰ N (as per Bunn & Boon, 1993) yielded a successful model output. This occurred because subtraction of all other enrichment values from the *P. australiensis* isotopic signatures failed to bring the shrimp within the confines of the convex polygon bounded by all primary sources, which is one of the primary assumptions required of the IsoSource

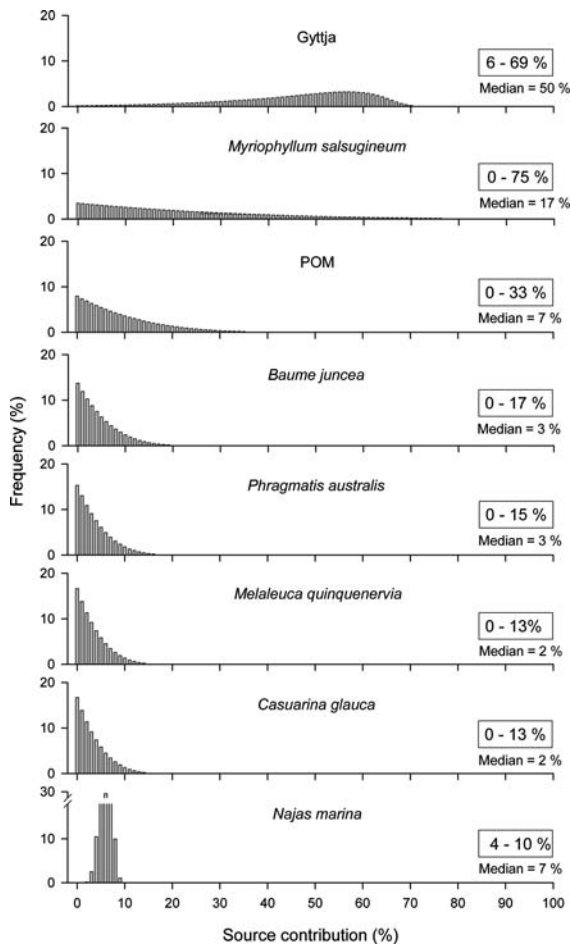
mixing model (for full explanation see Phillips & Gregg, 2003). Despite this, if we examine the model outcomes based upon the enrichment values from Bunn & Boon (1993), we see that the major contributing sources of *P. australiensis* diet identified by the mixing model were the same as those identified through interpretation of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values. For shrimp from control sites, the model predicted the macrophyte *M. salsugineum* and POM as being the major dietary components, contributing up to 54 and 72%, respectively (median values = 39 and 16%, respectively; Fig. 5). For gytija site shrimp, the model again identified *M. salsugineum* and POM as the highest contributors to *P. australiensis* diets (up to 75 and 33%, respectively), along with the important addition of gytija, which was predicted to compose anywhere between 6 and 69% (median = 50%) of shrimp diet at these affected sites (Fig. 6).

#### Discussion

This is the first study, to our knowledge, that has used stable isotope analysis to trace the assimilation of benthic cyanobacterial accumulations into an affected system. The  $\delta^{13}\text{C}$  values of *P. australiensis* from gytija-affected sites indicate they derive a substantial portion of their organic carbon requirements from the cyanobacterial accumulations. The  $^{13}\text{C}$ -enriched values of gytija and gytija-exposed shrimp sampled in 2003 suggest these decapods are assimilating gytija-derived C in combination with other primary sources more depleted in  $\delta^{13}\text{C}$ . Mixing models of *P. australiensis* isotope signatures from control and gytija locations further support these findings. The  $\delta^{15}\text{N}$  signatures of *P. australiensis* proved less informative than  $\delta^{13}\text{C}$  in elucidating difference in shrimp diet between gytija-affected and control habitats.

While there was little difference between the stable isotope signatures of shrimp from gytija-affected locations in 2002 and 2003, control shrimp in 2003 were slightly  $^{13}\text{C}$ -depleted and  $^{15}\text{N}$ -enriched over those from 2002. The major vegetation types found in Myall Lake, *Baume juncea* and *P. australis*, the leaf litter of *M. quinquenervia* and *C. glauca* and benthic detritus were all too depleted in  $\delta^{13}\text{C}$  to form anything but a small portion of the diets of *P. australiensis*. Similarly, the charophyte *N. marina*





**Fig. 6** Feasible contributions of primary food sources to the isotope signatures of *Paratya australiensis* from gytija-affected locations. Corrections for trophic enrichment have been made for  $^{13}\text{C}$  ( $-2\text{‰}$ ) and  $^{15}\text{N}$  ( $-5\text{‰}$ ). Values in boxes represent 1–99 percentile range of source contributions. Median (50 percentile) for each source contribution is also presented

was far too depleted in  $\delta^{15}\text{N}$  to be anything but a minor contributor. The likely major primary sources for the shrimp from all sites were POM and macrophytes (*M. salsugineum*), with the substantial addition of gytija at affected sites. Biofilms were not analysed as part of the current study, but have been found to be an important contributor to the carbon and nitrogen requirements of *P. australiensis* (Pringle, 1996; Burns & Walker, 2000). The stable isotope signature of biofilms sampled from Myall Lake several years after this study ( $\sim -22\text{‰}$  C and  $\sim 1\text{‰}$  N, Piola & Suthers unpublished data, Fig. 4) show it to be within the correct isotopic range to be a contributor to shrimp diets. When added to the

IsoSource mixing model calculations, this 2006 Myall Lake biofilm data are estimated to be a 0–44% (median = 9%) contributor to shrimp diets at reference sites, and a 22% (median = 4%) contributor at gytija-affected sites.

Interestingly, during 2003 sampling previously unobserved amounts of cyanobacterial accumulations were recorded at control site 2. These fine patches did not resemble the thick, dense mats of cyanobacteria seen at the gytija-affected locations, but rather small amounts of resuspended material that had been washed ashore through wind and wave action (an assumption supported by the high chl *a* levels recorded at this site in 2003; Methods). The  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of *P. australiensis* sampled from this site in 2003 appeared  $^{13}\text{C}$ - and  $^{15}\text{N}$ -enriched relative to shrimp values from the remaining control sites of that year, midway between control and gytija site values (Fig. 2). It appears likely that *P. australiensis* at site 2 had begun assimilating small amounts of the newly present cyanobacterial accumulations, with the C and N isotopic values of these shrimp in turn reflecting this new addition to their diet.

In general, cyanobacteria are assumed to be poor food for aquatic invertebrates (Porter & Orcutt, 1980) with low nutritional value (Ahlgran et al., 1992; Müller-Navarra, 1995). However this study showed it to have the best C:N ratio of all potential food sources. When mixed with other food sources containing additional essential elements (Burns & Hegarty, 1994), cyanobacteria may complement the diet of aquatic invertebrates (DeMott, 1998). Previous studies on the diets of atyid shrimp have shown active ingestion of cyanobacteria (Pringle, 1996; Burns & Walker, 2000). *Paratya australiensis* in the River Murray, Australia, were found to preferentially feed on non-toxic filamentous cyanobacteria (associated with biofilms), which formed up to 83% of the total fore-gut contents (Burns & Walker, 2000; Davie, 2002). *P. australiensis* were also able to successfully digest the cyanobacteria, which contrasted with findings for other crustacea (such as copepods) that pass large portions of cyanobacteria through their guts undigested (Burns & Xu, 1990). Findings from this study suggest that gytija is a major primary nutrition source for exposed atyid shrimp, providing between 6 and 69% of their dietary C and N. This conclusion is further supported by the positive relationship observed between gytija and

shrimp  $\delta^{13}\text{C}$ , with shrimp tissue becoming enriched in  $^{13}\text{C}$  in line with gyttja enrichment (Fig. 3).

While stable isotope analysis in this study supports the hypothesis that *P. australiensis* from gyttja-affected locations are feeding on cyanobacterial accumulations, the findings were not unequivocal. Mixing models required large shrimp trophic enrichment values to run successfully, and there are several possibilities as to why more conventional enrichment values (0–1.5‰ C and 2–3‰ N) were unable to resolve shrimp food sources. One likely reason is that a primary food source of *P. australiensis* was overlooked in this study. As previously mentioned, *P. australiensis* can derive substantial amounts of their organic carbon and nitrogen from biofilms (Pringle, 1996), which are often complex compositions of various food sources (e.g. algae, bacteria, microfauna, polysaccharides) that are selectively fed upon by the shrimp. Burns & Walker (2000) found the C and N isotopic values of cyanobacterial components within biofilms to be sufficiently high to explain the enriched *P. australiensis* values observed in this study (−24‰ for C and 7‰ for N). Secondly, microbial decomposition acting upon primary food sources (e.g. detrital matter and leaf litter) and the concurrent fractionation that occurs may lead to higher than expected  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values in grazers (Macko & Estep, 1984). Lastly, *P. australiensis* may naturally experience unusually high C and N isotopic enrichment above the signatures of their food sources. Trophic fractionation in aquatic food webs can be highly variable, ranging from −2.1 to 2.8‰ for  $\delta^{13}\text{C}$  and −0.7 to 9.2‰ for  $\delta^{15}\text{N}$ , with N-fractionation in particular being highest for herbivores and detritivores (of which *P. australiensis* is both) near the base of food webs (Vander Zanden & Rasmussen, 2001).

Use of the mixing model also demonstrated that a diet rich in POM or *M. salsugineum* could also result in the observed C and N isotopic signatures seen in *P. australiensis*. POM can have a highly variable isotopic signature (Piola et al., 2006) and it is possible the single isotopic “snapshot” of POM in this study may have underestimated its contribution to the diet of *P. australiensis*, particularly if *P. australiensis* is differentially ingesting specific food components masked within the greater POM pool. However, given *P. australiensis* from gyttja-affected sites were consistently enriched in  $\delta^{13}\text{C}$  and

$\delta^{15}\text{N}$  despite POM and other primary sources being isotopically uniform across all sites, it appears likely that shrimp from gyttja sites are assimilating some food source specific to these areas. Gyttja is the most probable source of this enrichment given its specificity to these locations and its enriched isotopic composition relative to other primary sources (particularly with respect to C).

Further laboratory-based testing is required to address questions and uncertainties raised from this study. Future research should include feeding experiments where *P. australiensis* from reference locations are reared on a diet of gyttja to determine if their isotopic signature becomes more similar to that of the cyanobacteria. Similar feeding studies could also elucidate the trophic enrichment values of *P. australiensis* across a range of diets. Finally, specific constituent components of food sources, such as cyanobacteria in biofilms or microalgae in POM, could be isolated and fed to *P. australiensis* to determine if enrichment values observed in this study may be the result of selective feeding upon sub-components within larger primary source pools.

The use of cyanobacterial accumulations as a food source by *P. australiensis* may not only include the transfer of C and N, but also the transfer of cyanobacteria hepatotoxins or related compounds. The majority of research on cyanobacterial toxicity has been conducted on planktonic blooms (Dasey et al., 2004); however, several instances of benthic toxic cyanobacterial accumulations have been reported (Mez et al., 1997; Hamill, 2001). Toxic assays on the cyanobacteria comprising the gyttja in Myall Lakes has shown the potential for the production of hepatotoxins and microcystin-type compounds (Dasey et al., 2004). *Paratya australiensis* in Myall Lake are eaten by a wide range of other organisms, and may act as an important transport vector for cyanobacterial toxins to higher trophic levels of both aquatic (e.g. macroinvertebrates and fish) and terrestrial (e.g. waterfowl and birds) food webs.

This study has demonstrated that gyttja-derived C and N are being assimilated into the local food web through *P. australiensis*; however, analysis of shrimp tissues is required to know whether possible hepatotoxins are also being transferred. A comparison of *P. australiensis* populations at gyttja-affected sites and unaffected locations is required to determine if consumption of toxic cyanobacterial accumulations is

affecting shrimp numbers and population dynamics. It may also be important to determine if any assimilated toxins accumulated by *P. australiensis* are being transferred to organisms higher up the trophic pathway, such as endangered bird species or commercially important fish and macroinvertebrate species.

**Acknowledgements** We thank S. Moore, K. Wright and J. Everett for field assistance and advice, and J. Wilson and our three referees for their helpful comments and many suggested improvements to the final manuscript. We also thank R. Diocares at Griffith University for all stable isotope sample analysis. This study was partly supported by funding from the New South Wales Department of Infrastructure, Planning and Natural Resources.

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