



Taxonomic, seasonal and spatial variation in the amino acid profile of freshwater macroinvertebrates

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Abstract

Macroinvertebrates play a key role in aquatic food webs, with amino acids (AA) playing an important role in determining their nutritional value to higher consumers. This study aimed to determine whether AA profile varies among four macroinvertebrate taxa, spatially and seasonally (summer/winter). The freshwater prawn, *Macrobrachium australiense*; freshwater shrimp, *Paratya australiensis*; freshwater snail, *Physa* sp. and caddisfly, *Triplectides* sp. were collected from seven sites along the Murray–Darling Basin, Australia. Sampling was conducted during summer 2015 (October–December), winter 2016 (May–June) and summer 2016 (October–December). AA profiles were found to be significantly different among the four taxa, with the highest total amino acid content found in decapods (*M. australiense* and *P. australiensis*). Based on the total essential AA content from our study, decapods had higher nutritional value compared to *Physa*. Seasonal variations in AA profiles of decapods were observed, with an increased proportion of the non-essential amino acid glycine and decreased proportion of different essential AAs found in individuals collected during winter compared to summer. No seasonal variation in the AA profiles of *Physa* sp. or *Triplectides* sp. was shown. Spatial variation in the AA profile of macroinvertebrates was only recorded in *P. australiense* during winter 2016. These findings expand the current understanding of the AA profile of freshwater macroinvertebrates showing that AA profiles are taxa specific and vary seasonally and spatially depending on taxa.

Keywords Macroinvertebrates · Amino acid · Nutritional value · Seasonal variation · Taxonomic variation · Water quality

Introduction

The nutritional value of prey items within a natural ecosystem can be assessed using a range of techniques, with amino acid (AAs) content being one of them (Vesterinen et al. 2020). Amino acids are one of the main biomolecules transferred across aquatic food webs (Dwyer et al. 2018). Apart from their role as building blocks of protein, they play a key role in cellular metabolism and function as energy metabolites (Vesterinen et al. 2020). The AA profile of an organism consists of 20 proteinogenic AAs from three domains: free

amino acids (FAAs), peptide AAs, and proteome AAs (Gu et al. 2015). Not all organisms can synthesise all AAs. Fish, for example, must obtain ten of twenty proteinogenic AAs from their diet (Li et al. 2009). Depending on organism's ability to synthesise AAs de-novo AAs are categorised into two groups—essential amino acids (EAA) and non-essential amino acids (NEAA) (Supplementary Table S1). A diet with a high proportion of EAAs is considered to have a higher nutritional value (Peres and Oliva-Teles 2006). For instance, fish must obtain lysine (Lys) (EAA) from their diet and as such Lys is considered as one of the major AAs that determine the nutritional quality of fish feeds (Kolmakova et al. 2013). More broadly, distributions of AAs in the diet of predatory fish have been reported to affect their metabolism, immunity, growth rate and fecundity (Rosa et al. 2005). Therefore a better understanding of the distribution and availability of AAs within a riverine ecosystem is important for assessing potential effects of resource depletion on different trophic levels.

The nutritional quality of different aquatic organisms to higher order consumers based on their AA profile has been

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studied previously (Chikaraishi et al. 2007; Kolmakova et al. 2013; Man et al. 2019; Pereira et al. 2013; Vesterinen et al. 2020; Yang et al. 2001). Nevertheless, studies on macroinvertebrates are still limited. Macroinvertebrates play a key role in the food web of aquatic ecosystems, forming a link between primary producers and higher order consumers (Bian et al. 2016; Paul et al. 2018) and have been suggested to contain a variety of different EAAs, making them a good quality food source for top predators such as fish (Bowman et al. 2019; Singh et al. 2017; Vesterinen et al. 2020). By comparison with marine invertebrates, studies on riverine macroinvertebrates are comparatively sparse (Dwyer et al. 2018; Thera et al. 2020). Understanding the AA profile (protein bound + FAA) of macroinvertebrates as prey items are, therefore, an important step towards understanding the availability of macronutrients to higher order consumers in riverine food webs. Such studies may also help to predict the ecological consequences of changes to the community composition of macroinvertebrates on the nutritional landscape (Dwyer et al. 2018).

The AA profiles of living organisms including macroinvertebrates have been known to have a conserved pattern defined by their taxonomy (Dwyer et al. 2018). Dwyer et al. (2018) showed that the AA profile of twenty aquatic insect taxa from six orders was taxa specific and correlated with phylogeny. Thera et al. (2020) and Vesterinen et al. (2020) also reported the taxonomic control on the AA profile of macroinvertebrates collected from different lakes. However, more studies are needed to improve present knowledge of species-specific AA variability in freshwater macroinvertebrates.

Intraspecific variation in AA profile has also been reported in various aquatic organisms (Aranguren-Riaño et al. 2018; Thera et al. 2020). Seasonal and spatial variations in environmental factors such as temperature, discharge, riparian shading, and water quality can alter the potential food sources for macroinvertebrates and cause biochemical changes within the organism such as changes in AA profile (Aranguren-Riaño et al. 2018; Boëchat and Adrian 2005; Leiwakabessy and Lewerissa 2017). In addition to this, unfavourable changes in the water quality itself can cause stress to macroinvertebrates leading to the up/downregulation of certain proteins and FAAs (Lane et al. 2019; Tomanek 2011). For instance, Binoy et al. (2012) reported spatial and seasonal differences in the AA profile of the fish species *Labeo gonius* collected from lentic and lotic water bodies. Intraspecific variability in AA profile has also been reported in different zooplankton taxa (Aranguren-Riaño et al. 2018; Ventura and Catalan 2010). However, seasonal and spatial variations in the AA profiles of macroinvertebrates in riverine ecosystems are not well understood (Thera et al. 2019, 2020). Moreover, the mechanisms that might drive these variations are largely unknown.

This study aimed to characterise the AA profile of four macroinvertebrate taxa to determine whether there is: (1) taxa specific variation in the AA profile of four taxa studied; *Macrobrachium australiense*, *Paratya australiensis*, *Physa* sp. and *Triplectides* sp. (2) seasonal variation in the AA profile of each taxon and (3) spatial variation in AA profile within each taxon. Addressing these knowledge gaps will allow us to better understand how season and location shape the potential nutritional value of aquatic macroinvertebrates as a food resource for consumers.

Methods

Study sites

Macroinvertebrates were collected from seven sites within the Murray–Darling Basin, Australia, from Jingellic in the upper Murray to Woods Point near where the Murray River enters Lake Alexandrina in South Australia, covering a vast geographic scale that is ideal for the study of spatial variations in the AA profiles of macroinvertebrates (Fig. 1). These sites are listed in Table 1 with details provided elsewhere (Paul et al. 2013, 2018). The sites vary widely in annual rainfall with the uppermost site (Jingellic) recording the highest annual rainfall (810 mm), the five intermediate sites (Yarrowonga, Euston, Lock 9, Burtundy, Murtho) are situated in the semiarid zone with an annual rainfall of approximately 260 mm and the lowest site (Woods Point) having a more temperate climate with a mean annual rainfall of approximately 400 mm.

Sample collection and study species

Macroinvertebrates were collected in summer 2015 (October–December), winter 2016 (May–June) and summer 2016 (October–December) as part of a long-term (but since discontinued) monitoring program for the Murray River (Paul et al. 2018). A combination of artificial substrate samplers (ASS) deployed for 6 weeks and sweep net sampling was used to collect the macroinvertebrates that colonized the substrate as well as those found in the major stream habitats such as: macrophytes, leaf packs and the water surface (Paul et al. 2013). Environmental variables including: water temperature (°C), pH, conductivity ($\mu\text{S cm}^{-1}$) and turbidity (NTU) were also recorded using a Yellow Springs Instruments (YSI) Pro DSS water quality meter (YSI Environmental, Yellow Spring, OH). Macroinvertebrates were identified to species level where possible and enumerated (Paul et al. 2013). Four taxa that represent key components of the food web were chosen for use in this study. These were: (1) a freshwater prawn, *M. australiense* (Decapoda: Palaemonidae), (2) a freshwater shrimp, *P. australiensis*

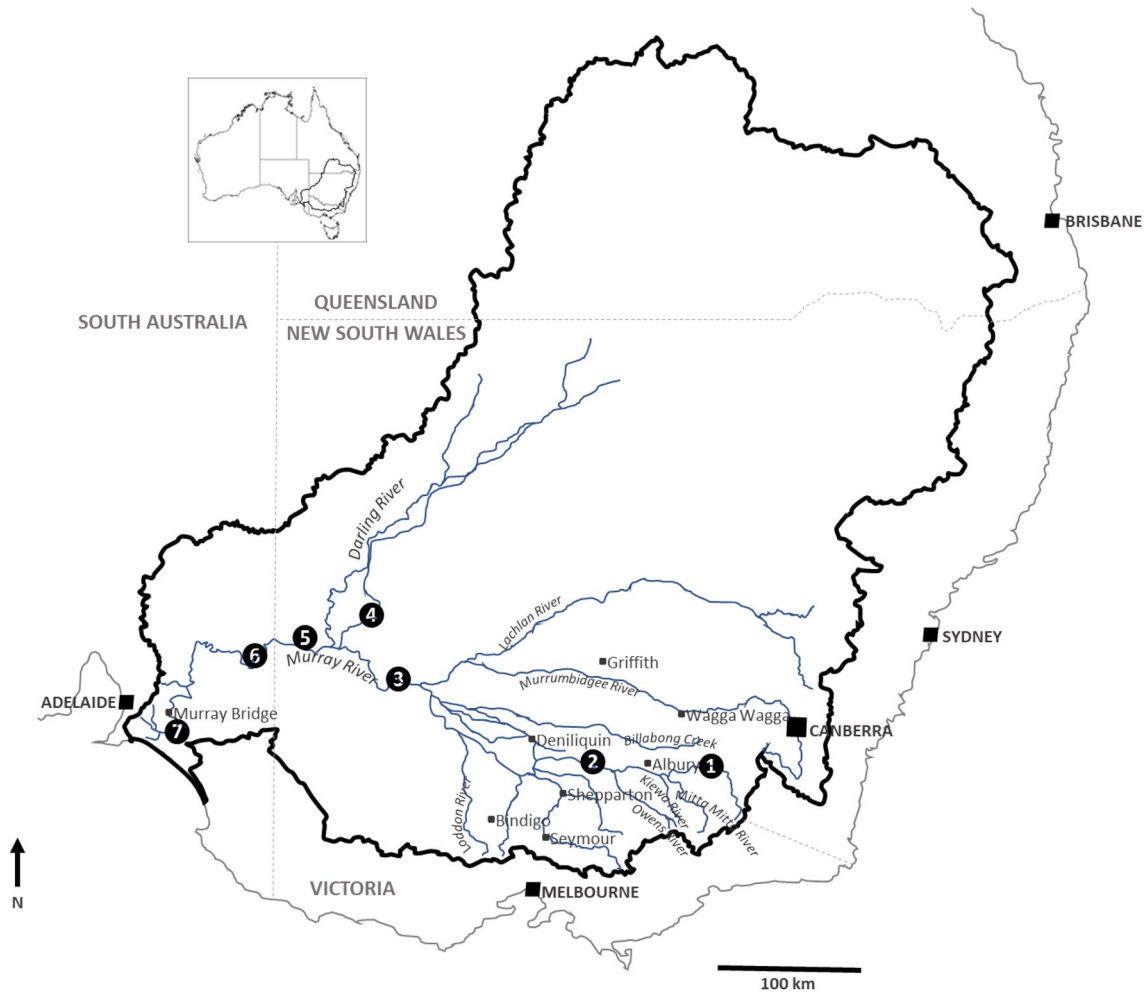


Fig. 1 Murray–Darling Basin in south-eastern Australia, showing the location of the seven sites used in this study: 1 = Jingellic, 2 = Yarrawonga, 3 = Euston, 4 = Burtundy, 5 = Lock 9, 6 = Murtho, 7 = Woods Point

Table 1 Details of River Murray Biological Monitoring program monitoring sites, modified from (Paul et al. 2018)

Site no.	Name	Latitude–longitude	Distance from the source (km)	Site description
1	Jingellic	S 35° 57.748' E 147° 30.517'	258	Below the junction of the Swampy Plains and Indi Rivers but upstream of the maximum extent of Lake Hume
2	Yarrawonga	S 36° 00.524' E 145° 57.571'	527	4 km below Yarrawonga Weir
3	Euston	S 34° 35.403' E 142° 45.190'	1389	3 km below of Euston Weir
4	Burtundy	S 33° 45.010' E 142° 15.580'	2607	On the Darling River, downstream of a small weir
5	Lock 9	S 34° 11.081' E 141° 36.204'	1737	1 km upstream of Lock 9 weir, lentic environment being within weir pool
6	Murtho	S 34° 06.8396' E 140° 81.1894'	1910	In the South Australian section of the River Murray, about 12 km north of Paringa
7	Woods Point	S 35° 13.966' E 139° 24.895'	2416	South Australian River section, about 16 km south of Murray Bridge at the end of Craton Lane

(Decapoda: Atyidae), (3) a freshwater snail, *Physa* sp. (Basommatophora: Physidae) and (4) a caddisfly, *Triplectides* sp. (Trichoptera: Leptoceridae). One to five specimens of each taxon from each site and season were analysed (Supplementary Table S2). *Physa* sp. and *Triplectides* sp. were collected only in 2016 summer and 2016 winter, whereas, the decapods (*M. australiense* and *P. australiensis*) were collected overall sampling periods.

In a preliminary study to test if gut contents contribute significantly to the overall AA profile of macroinvertebrates, 20 individuals of *P. australiensis* were sampled from the edge and benthic zones of a site at Wodonga creek. Ten individuals were frozen after transporting the samples to the laboratory, while the remaining 10 individuals were held overnight in river water to void their gut content prior to freezing (all specimens were frozen at $-80\text{ }^{\circ}\text{C}$). The AA profile of whole-body tissue of *P. australiensis* with and without gut contents was found to not significantly differ (PERMANOVA, pseudo- $F = 1.57$, $p = 0.2$) indicating that gut content does not significantly affect the AA profile of an organism (Supplementary Fig. S1); all subsequent AA analyses were conducted on whole organisms containing their gut contents.

Amino acid analysis

Sample processing and AA analysis broadly followed that described previously by Dwyer et al. (2018). Samples for AA analysis (whole animal) were first homogenized with Mini beadbeater-16 (Biospec) using approximately 0.2 g of 0.5 mm diameter glass beads (Biospec) in 1 mL of Milli-Q and then stored at $-80\text{ }^{\circ}\text{C}$. 100 μL of the thawed sample (10% of total dry biomass of individual sample) was freeze-dried in pyrolysed tubes ($550\text{ }^{\circ}\text{C}$) and hydrolysed with 6 N HCl containing 0.02% phenol at $110\text{ }^{\circ}\text{C}$ for 24 h under an argon atmosphere (Fountoulakis and Lahm 1998). After removing the acid in a rotary vacuum concentrator (RVC 2-18 CDplus; Martin Christ, Germany) at $40\text{ }^{\circ}\text{C}$ for 4.5 h, the AA mixture was reconstituted with 0.1% formic acid (using volume in $\mu\text{L} = 1,000,000 \times$ original weight of sample (g) in 1000 μL ; this step should give approximately 100 pmol/ μL of each AA). The AA mixture was then filtered through a 0.45 μm cellulose acetate membrane filter. 20 μL of the filtrate was buffered with 60 μL borate buffer (pH 9, Merck Centripur, Germany) and derivatized with 20 μL of 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC; Synchem UG & Co. KG) at room temperature. The tagged solution was then heated at $55\text{ }^{\circ}\text{C}$ (10 min) and diluted ($10\times$) with 0.1% formic acid prior to analysis.

Tagged AA samples were analysed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). The LC-MS/MS system consisted of a Shimadzu Nexera X2 UPLC coupled to a Shimadzu 8030 triple quadrupole mass

spectrometer, operated in positive ion electrospray ionisation (ESI) mode (Shimadzu corporation Kyoto, Japan). Individually tagged AAs were detected using multiple reaction monitoring (MRM), with collision parameters optimized individually. The separation was achieved using gradient elution (0.55 mL min^{-1}) through a Waters Aquity UPLC BEH C18 column ($2.1 \times 150\text{ mm}$; pore size 1.7 μm) maintained at $50\text{ }^{\circ}\text{C}$ (5 μL injection volume; 10 min run time). For the mass spectrometer the gas temperature, nebulizer gas flow, drying gas flow and interface voltage were set at $275\text{ }^{\circ}\text{C}$, 3.0 L min^{-1} , 17.0 L min^{-1} , 2.5 kV respectively. System control and data analysis were performed using LabSolutions software (Shimadzu, Tokyo, Japan).

Nineteen AAs quantified were: arginine (Arg), histidine (His), isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), phenylalanine (Phe), threonine (Thr), valine (Val), alanine (Ala), asparagine (Asn) + aspartic acid (Asp) as Asx, cystine (Cys-Cys), glutamine (Gln) + glutamic acid (Glu) as Glx, glycine (Gly), proline (Pro), serine (Ser) and tyrosine (Tyr). In order to identify and quantify these AAs, calibration standards were prepared from an amino acid standard H (Waters Corporation) spiked with Gln, Asn and Tryptophan (Trp), prepared at final concentrations in the range 0.01–2 pmol μL^{-1} . Reagent blank (20 μL of 0.1% formic acid, 80 μL Borate buffer and 20 μL AQC), sample blanks (Milli-Q treated as samples that went through the entire hydrolysis procedure) and 0.1% formic acid were also prepared for quality assurance (QA) along with quality control (QC) samples with selected AAs (His, Arg, Glu, Lys and Ile) to check peak positions and drift. Bovine Insulin (Sigma-Aldrich) was used as QC for the hydrolysis procedure with recoveries of each AA reported in Supplementary Table S3.

The AA profile for each sample is expressed as the relative abundance (mol%) of the amino acid pool. The total amino acid (TAA) content (mg per g dry biomass) in each sample was calculated by summing up the 17 AA masses (expressed as the polymerised molecular weights: Supplementary Table S1) obtained after acid hydrolysis (Lourenço et al. 2002). Similarly, the total essential amino acid (TEAA) content and total non-essential amino acid (TNEAA) content (mg per g dry biomass) were calculated from the sum of the 9 EAAs (Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Val and 8 NEAAs (Ala, Asx, Cys-Cys, Glx, Gly, Pro, Ser and Tyr) masses. During acid hydrolysis, Asn and Gln are deaminated to Asp and Glu, although they were specifically measured in this work to check for partial deamination. As is common practice, Asx (= Asn + Asp) and Glx (= Gln + Glu) are used to denote the combination of these AA pairs (Harris et al. 2016). Recoveries of Cys-Cys were very poor (< 30% in Bovine Insulin; BVI) but similar to those previously reported in the literature (Dwyer et al. 2018; Harris et al. 2016). Trp

cannot be measured in this procedure as it is destroyed during hydrolysis with hydrochloric acid.

Data analysis

Changes in the physical and chemical water quality parameters among sites during the three sampling periods were analysed by bar plots and principal components analysis (PCA). The relative abundance of each AA (expressed as mol%) were used in all statistical analyses. After testing for normality and homogeneity of variables using Shapiro–Wilk test, significant differences among species in regards to TAA content, seasonal difference in TAA content within each taxon were tested using one-way analysis of variance (ANOVA) followed by post-hoc Tukey's tests ($p < 0.05$). If the conditions for ANOVA were not fulfilled, a non-parametric Kruskal–Wallis's H test (Kruskal and Wallis 1952) was used ($p < 0.05$) in R (version 3.5.1). Bar plots and box plots were also made in R using the package ggplot2 (Wickham 2009).

All other statistical analyses were conducted using the PERMANOVA + V7.0.11 add-on to the Primer 7 statistical package (Anderson et al. 2008). Permutational multivariate ANOVA (PERMANOVA) was conducted to assess variation in AA profile between the four taxa and three seasons, using a two-factor design based on Euclidean distances. Permutation of residuals under a reduced model and Type III (partial) sums of squares type were used for PERMANOVA. When the main test showed significant differences between taxa and season, pairwise comparisons were performed within the PERMANOVA routine to determine taxa and seasonal differences (summer 2015, summer 2016 and winter 2016) in AA composition for each taxon. No significant seasonal difference was observed in the AA profile between two summers (summer 2015 and summer 2016) for *M. australiense* and *P. australiensis*. Hence, data from two summers (summer 2015 and summer 2016) were combined (as summer) for all the statistical analysis of TAA content, taxonomic variation and site variation in AA profile of these two taxa. Site variation in the AA profile of each taxon was also studied using PERMANOVA and pairwise analysis. Due to lack of adequate samples per site for *Triplectides* sp., variation in AA profile by site was not analysed for this taxon. The Mann–Whitney U test was used to check the significant seasonal variation in relative abundance of each AA per taxa.

Environmental variables were used as predictors in distance-based linear models' (distLM) and were fitted individually (marginal test) or together in AA (relative abundance) matrices data sets (sequential test) for each taxonomic profile (*M. australiense* and *P. australiensis* only). The Akaike Information Criterion (AIC) was used to establish the selection criteria, based on the specified selection procedure, to evaluate the 'best' model (for each

taxonomic group) that explains AA profile patterns and their responses to water quality. For visual interpretation of the models in multidimensional space, we used distance-based redundancy analysis (dbRDA) to generate ordination plots to illustrate associations between environmental variables and biological data (AA profile).

Results

Water quality of the sampling sites

Average water quality parameters of the seven sites at the time of macroinvertebrate collection during the three sampling periods are shown in Fig. 2a–d. Electrical conductivity (EC) in the Murray River gradually increased from upstream to downstream sites (site 1–7) and were all less than $500 \mu\text{S cm}^{-1}$. Salinity at the Burtundy site on the Darling River was noticeably higher with $\text{EC} > 500 \mu\text{S cm}^{-1}$.

Principal component analysis of the water quality data (Fig. 2e) shows a clear separation in water quality between summer and winter sampling. The first principal axis (PC1) explained 42.2% of the variation and was strongly correlated with temperature and pH. The second PC axis explained 32.5% of the variation and was correlated with EC and turbidity. The PCA also shows that seasonal variation in water quality within a site is higher than the spatial variation among sites. Within the sampling sites, Burtundy (site 4), which is the only sampling site from the Darling River is quite separated from all other sites along the Murray River due to higher EC and turbidity.

Total amino acid content

Macroinvertebrate taxa significantly varied in their TAA content [Kruskal–Wallis test, $H = 30.1$, $df = 2$, $p < 0.0001$ for summer; Kruskal–Wallis test, $H = 19.6$, $df = 2$, $p < 0.0001$ for winter; (Fig. 3)]. Among the three studied taxa, the TAA and TEAA content in *Physa* sp. was significantly lower than that of the two other taxa: *M. australiense* and *P. australiensis* (Table 2). The two decapods did not differ in TAA content. Data are not presented for *Triplectides* sp. due to the insufficient sample material for this taxon. The AA profile (mg per g of dry weight) is shown in Supplementary Table S5. No significant seasonal variation (summer and winter) were observed in the TAA concentration of *M. australiense* (Kruskal–Wallis test, $H = 3.02$, $df = 1$, $p = 0.08$), *P. australiensis* (Kruskal–Wallis test, $H = 0.80$, $df = 1$, $p = 0.37$) and *Physa* sp. (Kruskal–Wallis test, $H = 3.48$, $df = 1$, $p = 0.06$).

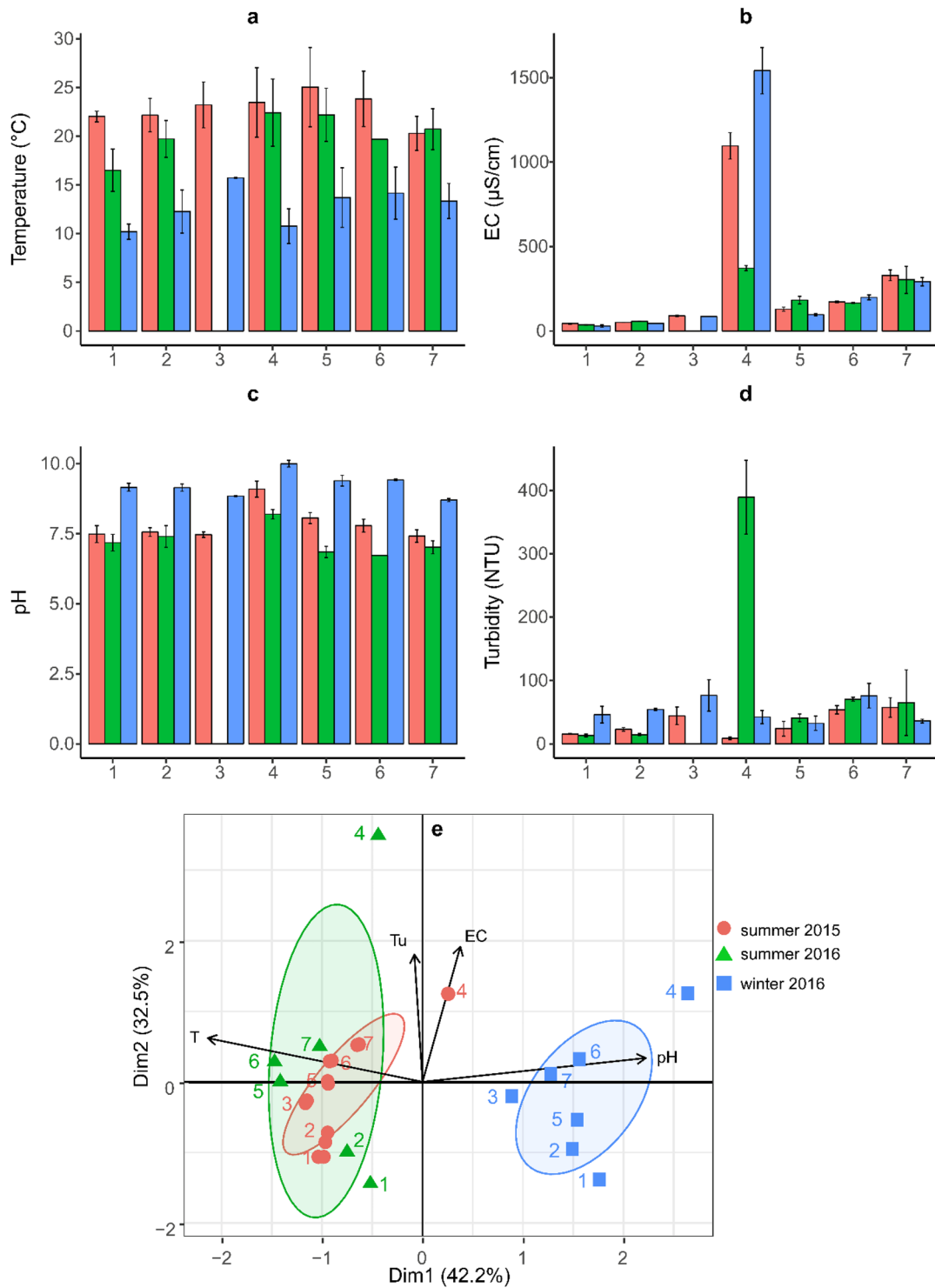


Fig. 2 Water quality parameters for each site measured during summer 2015, winter 2016 and summer 2016. Values represent the average (\pm SD) calculated from four measurements taken during each sampling period; two measurements were at the beginning of deployment of artificial substrate and two measurements were at the end of deployment (**a–d**). **e** Biplot of principal component analysis of water quality parameters at the seven samplings whereby the numbers rep-

resent sites: 1=Jingellic, 2=Yarrowonga, 3=Euston, 4=Burtundy, 5=Lock 9, 6=Murtho, 7=Woods Point and colours represent seasons: summer 2015 (filled circle), summer 2016 (filled triangle), winter 2016 (filled square). No data were recorded for Euston in summer 2016 and data was not available for the end of deployment period for Murtho in summer 2016. The measured variables are T: temperature (°C), EC: conductivity ($\mu\text{S cm}^{-1}$), pH, and Tu: turbidity (NTU)

Taxa specific amino acid profile

We quantified 17 AAs of which four NEAAs: Glx, Gly, Ala and Asx, were dominant in the four taxa studied (Fig. 4a, b). Met and His were the least abundant EAAs in all taxa. Comparing the relative abundance of TEAAs and TNEAAs, TNEAAs were relatively higher in all the studied taxa (Supplementary Table S4). A clear separation among the four studied taxa based on their AA profile was observed during summer (Fig. 4c) and winter (Fig. 4d), with AA profiles significantly different among the four taxa (summer: PERMANOVA, pseudo- $F=13.94$, $p=0.001$; winter: PERMANOVA, pseudo- $F=45.02$, $p=0.001$). The AAs Gly, Glx, Asx, Phe and Tyr were the major AAs that drove the differences among taxa.

Seasonal patterns of AA profile in macroinvertebrates

Intra-species variation in AA profile was also observed between seasons in some taxa (Fig. 5a) (PERMANOVA, pseudo- $F=8.7369$, $p=0.001$) with a significant interaction between taxa and sampling period (PERMANOVA, pseudo- $F=2.7972$, $p=0.004$). Significant intra-specific seasonal variations in AA profile of *M. australiense* and *P. australiensis* were observed, with winter 2016 samples significantly different from both summers (2015 and 2016) sampling periods. However, no seasonal variation in AA profile was observed for *Physa* and *Triplectides* sp. (Table 3). Furthermore, Gly, Leu and Val were shown to be the major AAs that changed in their proportions between seasons (summer and winter) in *M. australiense* (Fig. 5b, Supplementary Table S4) whereas, in *P. australiense*, seasonal variation was observed in Gly, Ser, Thr, Met, Lys, Ile and Pro (Fig. 5c, Supplementary Table S4). Both the decapods had a higher content of Gly in winter compared to summer (7% higher in *M. australiense* and 3% higher in *P. australiensis*).

The TEAA and TNEAA content for each taxon over different seasons are shown in Table 2. Substantial seasonal variations were observed in TEAA content in all studied taxa with less TEAA content during winter compared to summer.

Spatial variability of AA profiles

Amino acid profile of *M. australiense* showed no significant variation between sites over all seasons (summer 2015: PERMANOVA, pseudo- $F=0.86$, $p=0.61$; summer 2016: PERMANOVA, pseudo- $F=0.75$, $p=0.64$; and winter 2016: PERMANOVA, pseudo- $F=0.20$, $p=0.95$). Likewise, no spatial variation was observed in the AA profile of *Physa* sp. collected during summer and winter 2016, despite specimens being collected from four widely separated sites:

Woods Point (site 7), Murtho (site 6), Lock 9 (site 5) and Jingellic (site 1) (PERMANOVA, pseudo- $F=1.74$, $p=0.17$).

P. australiensis was collected from three locations [Woods Point (site 7), Murtho (site 5), and Yarrowonga (site 2)] during summer 2016 with no significant difference in the AA profile between sites (PERMANOVA, pseudo- $F=1.6$, $p=0.14$). Similarly, no site effect was detected in the AA profile of *P. australiensis* collected from all six sites along the Murray River during summer 2015 (PERMANOVA, pseudo- $F=1.01$, $p=0.31$). During winter 2016, *P. australiensis* was found only at three sites: Woods Point (site 7), Yarrowonga (site 2) and Jingellic (site 1) with significant spatial variation in the AA profile (PERMANOVA, pseudo- $F=14.46$, $p=0.001$) found. Pair-wise comparisons revealed that *P. australiense* from Woods Point (site 7) contained significantly different AA profile than specimens from Yarrowonga (site 2) ($t=4.4$, $p<0.05$) and Jingellic (site 1) ($t=3.4$, $p<0.05$); *P. australiensis* from Yarrowonga (site 2) and Jingellic (site 1) were not significantly different in their AA profile ($t=2.06$, $p=0.052$). Leu, Thr, Val, Phe, Ile, Tyr, Met and Cys–Cys were all found to be significantly lower in the Woods Point samples whereas Gly, Asx and Glx were significantly higher compared to the other two sites (Fig. 6).

Environmental predictors of AA profile

AA profile of decapods (*M. australiense* and *P. australiensis*) differed between the seasons (summer and winter), whereas, spatial variation was observed only for *P. australiensis* during winter 2016. The DistLM analysis with specified selection sequential tests of four environmental variables indicated that temperature and pH explained 51.3% of the variation in AA profile of *M. australiense* based on the AIC values (Table 4 and Fig. 7a). In the case of *P. australiensis*, temperature and turbidity were the only two significant environmental parameters (Table 4), explaining around 23.6% of the variation in the AA profile (Fig. 7b). Temperature was the most important single environmental predictor of decapod AA profiles (34.7% and 17.8% of the variation in the AA profile in *M. australiense* and *P. australiensis*, respectively).

Discussion

Variation in total amino acid content among taxa

Overall, in our study decapods were found to contain a higher TAA content compared to the snail indicating their high nutritional value. Higher protein concentration in decapods compared to *Physa* could be due to their omnivorous feeding habit and also may be due to decapods containing more muscle tissue than exoskeleton (Abdel-Salam 2014).

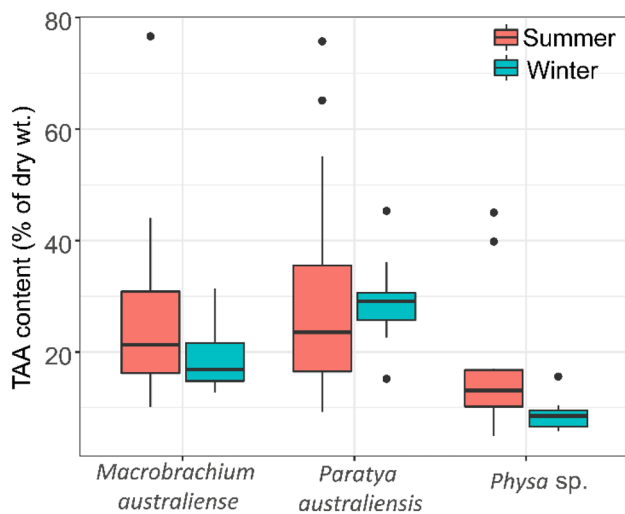


Fig. 3 Total amino acid content (TAA) in *Macrobrachium australiense*, *Paratya australiensis*, and *Physa* sp. during summer (2015 and 2016 combined) and winter seasons

Zukowski and Walker (2009) reported that *Physa acuta* is the most abundant freshwater snail in the lower River Murray. *Physa acuta* is an introduced species and their ascendancy has been linked to the decline of native species (Zukowski and Walker 2009). Given the differences in nutritional quality between the snail *Physa* sp. and the other taxa in this study, the increasing population of this species could affect the availability of TAA/protein to higher order consumers in the Murray River.

AA profiles in macroinvertebrates are taxa specific

In line with findings from previous studies (Aranguren-Riaño et al. 2018; Dwyer et al. 2018), AA profiles successfully discriminated between the four taxa (*M. australiense*, *P. australiensis*, *Physa* sp. and *Tripletides* sp.) regardless of having been collected from a wide geographic range and across various seasons. This supports the assumptions made by Bogatyreva et al. (2006) and Dwyer et al. (2018) that AA profile in living organisms is a highly conserved pattern and controlled by genetic information.

Apart from Ala, the proportion of all other (16) AAs differed among taxa. Differences in AA profile among

macroinvertebrate taxa also suggest that streams and rivers with a diverse macroinvertebrate community structure may provide a wider variety of AAs for top consumers than rivers with lower diversity. In addition to this, changes in community composition of macroinvertebrates will alter the nutritional landscape available to top predators such as fish (Dwyer et al. 2018). Previous studies have reported that diets higher in EAAs compared to NEAAs lead to better development and protein synthesis in fish (Peres and Oliva-Teles 2006). Based on the TEAA content from our study, decapods had higher nutritional value compared to *Physa* sp. A higher amount of TEAA in decapods has also been reported in previous studies (Bhavan et al. 2010; Yanar and Çelik 2006). The EAA-Val was found in the highest proportion in *Tripletides* but this taxon contained a lower proportion of other EAA such as Leu, Phe and Met. Among the nine EAAs, the abundance of Met and His are lowest in all the studied taxa. Many studies have found that Met and His are the limiting EAA for fish and other higher consumers feeding on macroinvertebrates (Reed and D'Abramo 1989; Yanar and Çelik 2006). Glx, Ala, Asx and Gly were found to be the dominant NEAAs in the studied macroinvertebrates which were in accordance with the findings from previous studies (Çagiltay et al. 2011; Leiwakabessy and Lewerissa 2017; Reed and D'Abramo 1989).

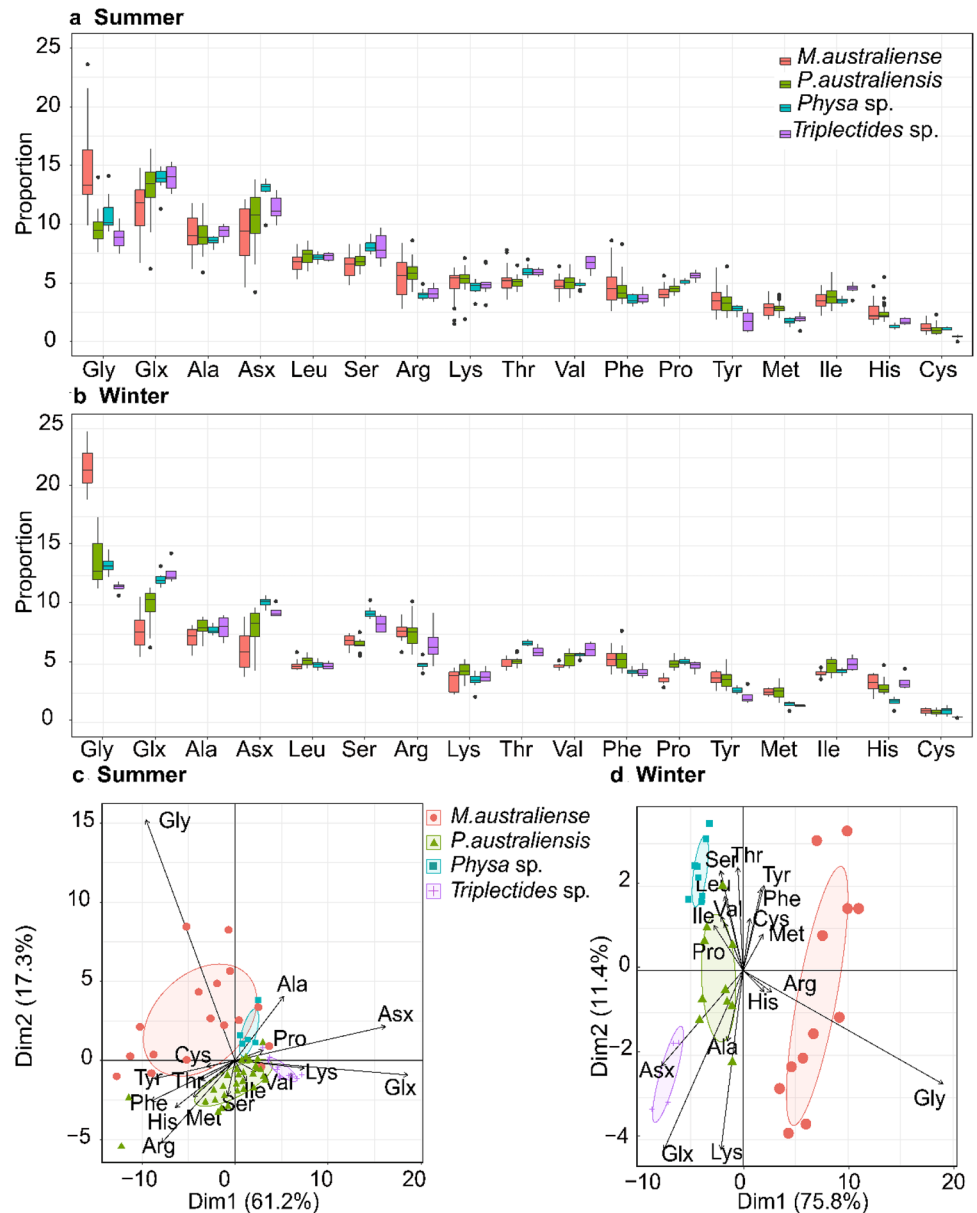
AA profile in macroinvertebrates with respect to seasons

Many studies have emphasized season as one of the major factors that shape the AA profile of organisms (Binoy et al. 2012; Çagiltay et al. 2011; Çaglak and Karsli 2017; Ghribi et al. 2018). However, the study of food webs in rivers and streams are often limited to a single point of time, which makes our understanding about riverine nutritional ecology incomplete. Given rivers are dynamic systems, interactions between different trophic levels and energy flow could be affected by seasonal changes in food sources or food nutritional quality. Our results show that even though there is no significant seasonal change in the TAA content of macroinvertebrates, the AA profile of the two decapod species *M. australiense* and *P. australiensis* show significant seasonal variation; *Physa* sp. and *Tripletides* sp. on the other hand, showed no such variation.

Table 2 Total essential amino acids (TEAA) and Total nonessential amino acids (TNEAA) in three macroinvertebrate taxa expressed as mg per g dry weight, across the two seasons

Taxa	Summer			Winter		
	TEAA	TNEAA	TAA	TEAA	TNEAA	TAA
<i>Macrobrachium australiense</i>	117.3±5.7	138.0±7.4	255±12.9	95.9±2.7	98.3±3.7	194±6.2
<i>Paratya australiensis</i>	129.4±6.2	148.1±8.0	278±12.9	144.4±3.4	143.5±3.7	288±6.9
<i>Physa</i> sp.	74.4±5.6	104.5±8.1	179±13.7	39±1.3	51±1.8	90±3.17

Fig. 4 The amino acid profiles (relative abundance expressed as mol%) for *Macrobrachium australiense*, *Paratya australiensis*, *Physa* sp. and *Triplectides* sp. during: **a** summer (2015 and 2016 combined) and **b** winter (2016). Also shown are Principal component Analysis (PCA) of the amino acid profiles of the four macroinvertebrate taxa over two seasons: **c** summer (2015 and 2016 combined) and **d** winter (2016) with vectors shown for amino acids (Cys: cystine)



The changes in the AA profile of decapods with season is consistent with a previous study on the mussel *Mytilus edulis* (Li et al. 2015). The nutritional value of decapods is higher during summer, with respect to their TEAA content. For *M. australiense* the EAAs Ser, Val, Ile decreased in winter compared to summer while Gly increased. Similarly, for *P. australiensis* the EAAs Lys, Ser, Thr, Met decreased during winter while Ile and Gly increased. Lys and Met are considered to be important indicators of the nutritional value of fish diets (Li et al. 2009). Our results showed a significant decrease in the Lys and Met levels in winter compared to summer in *P. australiensis*. A decrease in Lys content was also observed in *Physa* sp. in winter even though the overall AA profile did not significantly differ. A decrease in the availability of different EAAs in macroinvertebrates during

winter may result in a poorer quality diet with decreased nutritional value for higher consumers like fish. Top predators therefore may need to adopt different feeding strategies to meet their AA requirements across seasons. This highlights a need to assess the transfer of EAAs between macroinvertebrates and their predators to understand the effects of fluctuating EAA contents on growth and metabolism of higher order consumers in a riverine environments.

Seasonal changes in water temperature can change the basal food resources in rivers. One reason for the changes in the AA profile of decapods during summer and winter could be the result of varied food resources available to the decapods during the two different seasons. The similarity of AA profile within two summer sampling periods (summer 2015 and summer 2016) in *M. australiense* and *P.*

Fig. 5 a Multidimensional scaling (MDS) ordination based on 95% confidence interval bootstrap average showing seasonal differences in the amino acid profile of *Macrobrachium australiense*, *Paratya australiensis*, but not *Physa* sp. or *Triplectides* sp. collected over three sampling periods (summer 2015, summer 2016 and winter 2016). Note *Physa* sp. and *Triplectides* sp. were not sampled during summer 2015. Black symbols represent average location for particular groups. Principal component analysis plots showing seasonal differences in amino acid profile for **b** *Macrobrachium australiense* and **c** *Paratya australiensis* (summer 2015 and summer 2016 combined); (Cys: cystine)

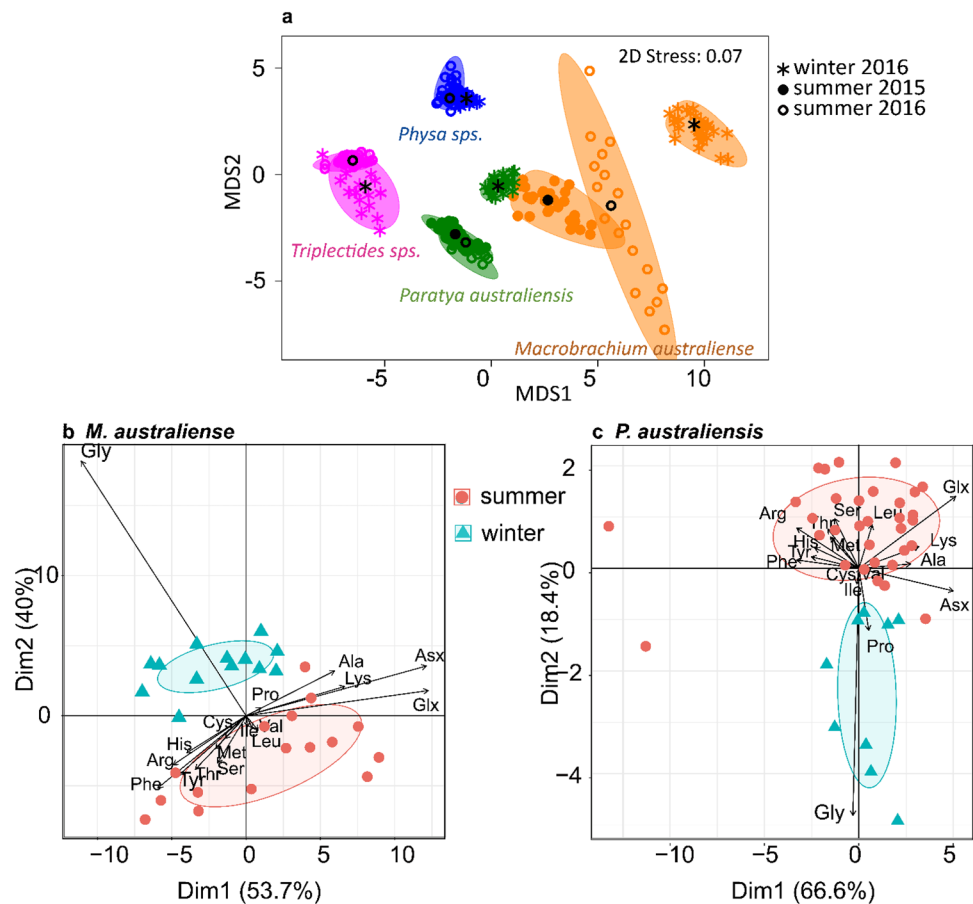


Table 3 Permutational multivariate ANOVA (PERMANOVA) pairwise comparisons for macroinvertebrate amino acid profile using ‘taxa’ and ‘season’ as fixed factors

Taxa	Groups	<i>t</i>	<i>p</i> (perm)	Unique permutations
<i>Macrobrachium australiense</i>	Winter 2016–Summer 2015	4.16	0.001	999
	Winter 2016–Summer 2016	2.16	0.018	819
	Summer 2015–Summer 2016	0.97	0.335	772
<i>Paratya australiensis</i>	Winter 2016–Summer 2015	2.52	0.001	998
	Winter 2016–Summer 2016	2.15	0.003	999
	Summer 2015–Summer 2016	0.60	0.696	999
<i>Physa</i> sp.	Winter 2016–Summer 2016	1.30	0.161	708
<i>Triplectides</i> sp.	Winter 2016–Summer 2016	0.96	0.451	773

p-values in bold correspond to significantly different amino acid profiles (*p* < 0.05)

australiensis provide some support for this idea. Decapod diets generally consist of a wide variety of foods such as biofilms, littoral plants and fine particulate organic matter (Burns and Walker 2000). Seasonal variation in discharge, temperature and shading of the riparian region in rivers can alter food resources for decapods through changes in basal community composition, productivity or changes in food web (Torres-Ruiz et al. 2007). Such variation in diet might be a reason for seasonal intraspecies variation in the AA

profile of decapods. However, previous studies suggest that diet may not be an influencing factor defining the AA profile of organisms. Brückner et al. (2017) showed diet did not play a role in defining the AA profile of oribatid mites (microarthropods). These soil microarthropod’s feeding habits are diverse, ranging from decomposers to scavengers and predators, but their AA profile was similar despite significant differences in the AA profile of their food. Moreover, none of the measured AAs correlated with the AA profile

Fig. 6 Amino acid profile (relative abundance expressed as mol%) in *Paratya australiensis* collected from three sites along the Murray River during winter 2016 (site 1: Jingellic, site 2: Yarrowonga, site 7: Woods Point); (Cys: cystine)

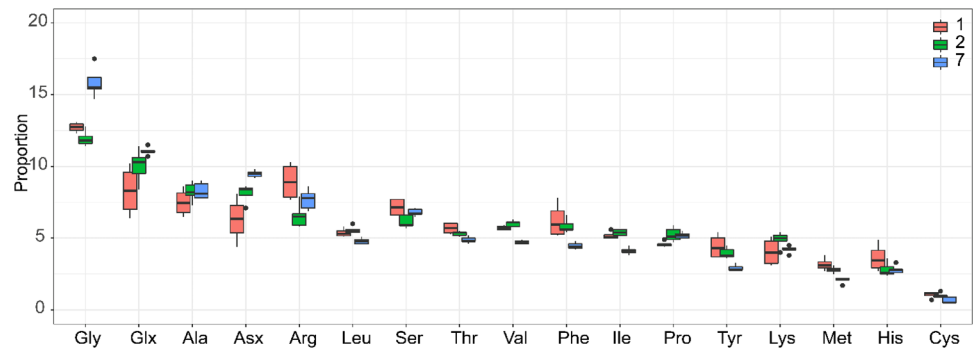
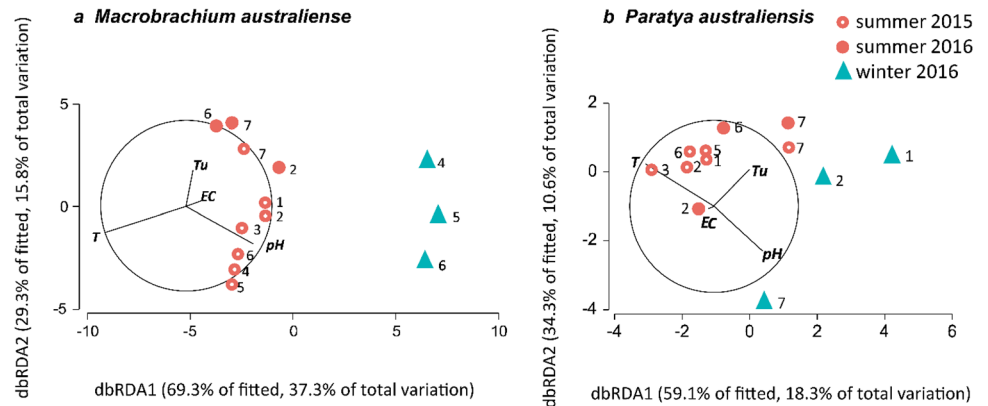


Table 4 DistLM results of amino acid profile for *Macrobrachium australiense* and *Paratya australiensis* against 4 predictor water quality variables (999 permutations). Bold indicates variables significantly correlated with amino acid profile at $p < 0.05$

Predictor variable	Taxa	Marginal test			Specified selection sequential tests			
		Pseudo- <i>F</i>	<i>p</i>	Proportion variation explained	Pseudo- <i>F</i>	<i>p</i>	Proportion variation explained	Cumulative variation explained
Temperature (°C)	<i>M. australiense</i>	24.45	0.001	34.7	24.45	0.001	34.7	34.7
	<i>P. australiensis</i>	8.67	0.001	14.8	8.67	0.003	14.8	14.8
pH	<i>M. australiense</i>	16.07	0.001	25.9	15.22	0.001	16.6	51.3
	<i>P. australiensis</i>	4.98	0.014	9.1	0.64	0.454	1.1	15.9
Turbidity (NTU)	<i>M. australiense</i>	1.63	0.181	3.4	1.25	0.266	1.3	52.6
	<i>P. australiensis</i>	3.06	0.046	5.7	4.58	0.025	7.3	23.2
Conductivity (µs/cm)	<i>M. australiense</i>	0.57	0.579	1.2	0.43	0.667	0.6	53.2
	<i>P. australiensis</i>	0.36	0.766	0.7	0.81	0.390	1.3	24.5

Fig. 7 Distance-based redundancy analysis (dbRDA) of Euclidean distance matrix calculated from standardised amino acid data for: **a** *Macrobrachium australiense* and **b** *Paratya australiensis* showing water quality parameters (T: temperature, Tu: turbidity, EC: conductivity and pH) as predictor variables. Numbers represent sites: 1 = Jingellic, 2 = Yarrowonga, 3 = Euston, 4 = Burundy, 5 = Lock 9, 6 = Murtho, 7 = Woods Point



of these resources. Srivastava et al. (2006) reported that, despite feeding the rotifer *Brachionus plicatilis* on five varieties of food, this caused no changes to their AA profiles even though the food differed in AA profile. Similarly, Boëchat and Adrian (2005) showed that two species of freshwater ciliate have significantly different AA profiles despite feeding on the same algae as a food resource. Hence, further evidence would be required to support the idea that changing diet is a major factor behind seasonal and temporal variations in the AA profiles of the decapods studied here.

We also showed that the AA profile of purged and unpurged shrimp was not found to be different, suggesting that gut content does not significantly affect the AA profile of decapods. Therefore, the seasonal variation in AA profile of decapods observed in our study is not due to differences in the gut content of the decapods at time of sampling. The lack of effect of gut contents on AA profile is likely due to the relatively small contribution of the gut compared to the whole-body mass.

Another possible reason for the seasonal variation in the nutritional quality of decapods could be due to changes in

the biochemical response (AA or protein) of organisms triggered by changes in water quality parameters driven by season. Water quality parameters, especially temperature and pH, differed substantially between seasons (Fig. 2). DistLM results showed around 53% of AA profile variation of *M. australiense* and 24% of AA profile variation in *P. australiensis* are explained by the water quality parameters, with temperature alone explaining the most seasonal and spatial variation (34% and 15%, respectively). Water temperature is the main abiotic driver that influences feeding, growth, behaviour, life cycle and metabolism in macroinvertebrates (Dallas and Rivers-Moore 2012; Li et al. 2013). Previous studies have demonstrated that temperature changes alter the FAA content in different organisms like adult barnacle *Balanus balanoides* (Cook et al. 1972); white shrimp, *Litopenaeus vannamei* (Zhou et al. 2011); and the beetles *Sitophilus granarius* and *Cryptolestes ferrugineus* (Fields et al. 1998). Further investigation is required to determine the influence temperature has on determining the AA profile of freshwater organisms and thus potential for altering the nutritional landscape of aquatic ecosystems. This is greatly needed given the threat of climate change and anthropogenic changes to temperature regimes within aquatic ecosystems through cold water pollution associated with river regulation.

The greatest seasonal variation in AA profile was seen in the Gly content with a difference of around 7% in *M. australiense* and 3% in *P. australiensis*, with a higher content during winter. Gly is synthesized from Ser and Thr (Wang et al. 2013; Xie et al. 2014). Decreases in Ser and Thr were also shown between summer and winter suggesting that the up-regulation of Gly in winter might have decreased the amount of Ser and Thr (Supplementary Table S4) in decapods during this season. In contrast, previous studies have shown that Gly content (Protein bound + free) decreased in winter compared to summer (Yanar and Çelik 2006). This has also been shown for other decapods such as the green tiger shrimp and speckled shrimps from the eastern Mediterranean coast (Yanar and Çelik 2006), red shrimp, pink shrimp and Norway lobster (Rosa and Nunes 2004). According to Zhou et al. (2011), response of individual AA to temperature depends upon the species. Even though Gly is one of the NEAAs for macroinvertebrates and fish, it is known to participate in gene expression and regulation and has a role in the immune response of fish. Gly has also been reported as an important AA in the osmoregulatory responses of aquatic animals to environmental stress (Xie et al. 2014) by improving oxidation resistance capacity. They reported that increases in Gly in the diet of shrimps significantly increased the survival of shrimps exposed to low salinity with increases in growth rate, protein production as well as whole body concentrations of Mg, Ca and Fe (Xie et al. 2014). The change in Gly content therefore might

also reflect a protective mechanism in response to changing temperatures in the decapods in this study.

Spatial pattern of AA within taxa

No variation in AA profile of *M. australiense*, *P. australiensis* (winter is an exception) and *Physa* sp. collected from different sites along the Murray River was found in this study despite the EC and turbidity changes along the Murray River. Conductivity varied from 24 to 373 $\mu\text{S cm}^{-1}$ at sites along the Murray and was substantially higher at Burtundy (Darling River) ranging between 359 and 1660 $\mu\text{S cm}^{-1}$. Such variation in water quality among sites, however, seems to have less impact on the AA profile of macroinvertebrates compared to the seasonal factors and shows macroinvertebrates may be able to maintain AA homeostasis in a wide range of habitats. In contrast, various studies have shown that salinity impacts on the FAA profile of decapods due to role of FAA (Glu, Ala, Gly, Arg, Pro and Lys) in osmoregulation (Koyama et al. 2018; Yang et al. 2001). Yang et al. (2001) reported a relationship between salinity and changes in AAs of freshwater prawns however the effects on FAAs were seen at much higher salinity concentrations than what were present at sites in this study.

Even though site variation was not observed in the AA profile of *P. australiensis* collected from six sampling sites (Murtho, Woods point, Yarrawonga, Lock 9, Jingellic, Euston) in summer 2015 and three sampling sites (Murtho, Woods point and Yarrawonga) in summer 2016, there was a significant difference in the AA profile of *P. australiensis* collected from three sites (Woods point, Yarrawonga and Jingellic) in winter 2016. All the EAAs except Arg, His and Lys were found to be lower in the shrimps from Woods Point compared to that of the upper two sites (Yarrawonga and Jingellic). DistLM analysis showed that temperature and EC explained around 24% of the variation in the AA profile of *P. australiensis* between sites during winter. Marie et al. (2017) also reported changes to the transcriptome in the shrimp *Plalemon carideans* due to the combined stress of temperature and salinity which is likely to have altered the AA profile of this taxa. Another possible explanation for the observed spatial variation could be due to possible genetic differences in the population of *P. australiensis* between upstream and downstream of the Murray River. However, this is less likely given no site difference was shown during summer. The observed site variation in AA profile of *P. australiensis* during winter may be attributed to other factors like ontogeny (Roustaian et al. 2000) or sex (Bhavan et al. 2010). Further research is thus needed to tease out the factors contributing to spatial variations in AA profiles of *P. australiensis* during winter.

Conclusion

This study shows that the AA profile of freshwater macroinvertebrates is not only taxon specific but can vary temporally (between seasons) and spatially between sites depending on taxa. A change in the community composition of macroinvertebrates can therefore potentially alter the nutritional landscape available to higher order consumers within riverine environments. Further research is required to determine the relative influence of seasonal and spatial variation in AAs due to inter and intraspecific differences, on the growth and metabolism of top predators and to understand the cascading effects across the food web.

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Availability of data and material The data that supports the findings of this study are available from the corresponding author upon request.

Compliance with ethical standards

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

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