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MMCP Collaboration Final Report 2019 - Understanding the ecological consequences of macroinvertebrate community structure change

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The MMCP Collaboration Final Report 2019 – Understanding the ecological consequences of macroinvertebrate community structure change

Final Report prepared for the Murray–Darling Basin Authority.

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Background

The initial aims of this theme were to: 1) build on the understanding that had derived from the long-term monitoring program of macroinvertebrates in the Murray River, and 2) advance the understanding and application of knowledge of nutritional ecology in freshwater systems. The 35-year monitoring program of macroinvertebrates in the Murray River (currently the River Murray Biological Monitoring Project (RMBMP)) has developed an extensive dataset that could improve our knowledge of the functional role of macroinvertebrates in rivers more generally. Previous work has shown a shift has occurred in overall macroinvertebrate community structure in the Murray River over time (Paul *et al.* 2018). Advanced modelling techniques now allow interrogation of long-term data sets and investigate whether environmental variables (including flows) can explain any of the long-term variations within macroinvertebrate communities.

Food quantity and quality are just two elements that can be important in determining community structures, thus influencing top predators such as fish. Since macroinvertebrates represent one form of prey item for consumers, a change in the type of prey items, due to the change in macroinvertebrates, could mean: 1) that the overall energy available for consumers may have altered, and/or 2) the nutritional landscape for consumers also changes. The amount of energy available can be measured in terms of the total biomass available as prey. However, the amount of food available may not alone be a limiting factor. The basic tenant in nutritional ecology theory suggests that if the nutritional landscape for a given animal is sub optimal, there will be a metabolic cost for it to achieve its optimal diet. Such a metabolic cost is likely to be reflected in a range of biological functions of the organism, e.g. reduced growth rates and, fecundity. Emerging research is now showing that aspects of nutritional ecology may be important in structuring riverine communities, and that improved nutritional status of river systems may be an important outcome of environmental watering (Guo *et al.* 2016).

Yabbies (*Cherax destructor*), along with other decapods, are a major source of food for fish (Ebner 2006; Stoffels 2013). Yabbies are omnivorous, deriving food from a range of detrital and protein food sources (Duffy *et al.* 2011; Giling *et al.* 2009; Giling *et al.* 2012), making them an important linking part of the riverine food web (Giling *et al.* 2009). Small but significant changes can occur in the C:N ratio of yabbies in response to different riparian vegetation (Giling *et al.* 2012), so any environmental flow that can mediate a changed environment could likely lead to similar changes in the nutritional value of yabbies.

To address the key background issues, three overarching questions were addressed by this component of work:

Can flow variables be used to predict the quantity and quality of food resources in rivers?

Do primary food resources alter the growth rates and nutritional quality of key fish prey?

Does floodplain connectivity lead to any improved nutritional quality of key fish prey?

To address these three questions, a review of the MMCP stocktake report supported the progress of three pieces of work:

1. Using the River Murray Biological Monitoring Project data as an example of a long-term monitoring data set to determine whether general inferences can be made about how the number (biomass) of taxa, taxa richness and overall nutritional value of benthic invertebrates have changed over time. If changes have occurred, then a secondary aspect is to determine whether any general flow characteristics explain the changes.

2. Using decapods as target taxa to determine whether their nutritional value is enhanced though connection with floodplains.

3. Using laboratory trials to determine the growth of a representative decapod (yabby) in response to three diets for differing quality.

Each of these broad activities involved a number of tasks and each are considered separately.

Macroinvertebrate responses to environmental variables

Background

A healthy and productive fish community relies on the underlying food web to provide all of its energetic and nutritional needs; an understanding of food quantity and nutritional ecology sit at the core of these food webs.

Ecological stoichiometry (ES) and the Geometric framework (GF) are two well-established theoretical frameworks that have been used to examine nutritional ecology. ES examines the flow and balance of key elements (generally carbon, nitrogen and phosphorus) through prey and consumers (Elser *et al.* 2000). Thus, such an approach examines how the various C, N and P ratios can drive the ecology of prey and consumers. On the other hand, the GF takes multiple nutrients into consideration and can include macro nutrients such as protein, lipids as well as their monomeric nutrients such as amino acids and fatty acids (Raubenheimer *et al.* 2009; Simpson & Raubenheimer 1993). Four principles have been recognised that underpin nutritional ecology (Stoffels 2013):

- Prey species differ in their nutrient composition and therefore, their nutritional value to consumers.

- Consumers have a characteristic stoichiometry, defined either by their genetics/physiology or behaviour.

- Maintaining a balanced stoichiometry may incur a cost to fitness to consumers.

- Variation in the stoichiometry of consumers means variation in the overall consumer needs.

Thus, a 'balanced diet' is critical to maintaining healthy individuals and populations. Diets with balanced amounts of macronutrients are known to be important in fish communities. The aquaculture industry has invested significant effort examining the food requirements for fish, optimizing factors such as specific protein requirements (De Silva *et al.* 1989), protein:energy ratios (De Silva *et al.* 2002) and responses to various protein sources (Abery *et al.* 2002).

Australian context

Macroinvertebrates are central to riverine food webs. The 35-year monitoring program of macroinvertebrates in the Murray River (the RMBMP, funded by the MDBA) has examined macroinvertebrate communities in the Murray River over time and at different sites. Any environmental factors driving changes in the Murray River that have led to alterations in

macroinvertebrate communities, either abundances of all or specific organisms and/or community composition, may have altered the nutritional landscape available for native fish.

The study of fish nutrition has often been examined for those species that are being reared for artificial propagation (e.g. salmonids) and accordingly, study has tended to examine the broadest nutritional aspects. Nutritional studies of Australian native fish have often occurred for similar reasons. For example, determining relative benefits of growing Murray cod (*Maccullochella peelii* Mitchell, 1838) on blood meal or soybean meal (Abery *et al.* 2002). It should be noted that there are reasonably few examples of nutritional examination of Australian native fish and consequently, the nutritional ecology of Australian freshwater fishes is not as well understood. In particular, little is known about whether a changed nutritional landscape, brought about by changes in prey, has implications for healthy and productive fish communities in riverine systems.

Aim

The aim of this component was to use the understanding derived from the monitoring program as a basis for understanding the ecological consequences of changes to potential food resources in rivers. Our experimental design took the following approach:

1. Aggregate all macroinvertebrate data from the RMBMP.

2. Examine community composition across all sites over time.

3. Generate a refined dataset of the number of benthic macroinvertebrates at each site over time.

4. Generate conversion factors to translate numbers of organisms into biomass.

5. Interrogate existing databases to determine carbon:nitrogen (C:N) ratios of macroinvertebrates.

6. Examine species richness, biomass and C:N ratio over time as a function of a suite of flow variables.

Methods

Data collection and primary analysis

The overall macroinvertebrate database was derived from the RMBMP data set. Briefly, macroinvertebrate sampling was conducted twice a year — once in winter (May–June) and once in summer (October–December) at sites along the Murray River (Table 1). Macroinvertebrates were collected from artificial substrates previously deployed at each of the sites and the identity of the animals was confirmed upon return to the laboratory.

The community composition data determined at each site, from 1980 to 2015, were compiled in a single database, thus giving an historical account of community structure over time. Multivariate statistical analysis was used to generate visual ordinations of community responses.

Table 1. Sites (name and number designation) routinely sampled as part of the Murray River Monitoring Project.

Site no.	Location /site name (bold)	Latitude and longitude
801	Downstream of **Jingellic**	S 35°57.748' E 147°30.517'
804	Downstream of **Yarrawonga Weir**	S 36°00.524' E 145°57.571'
808	Downstream of **Euston Weir**	S 34°35.403' E 142°45.190'
811	Upstream of **Lock 9** at Cullulleraine	S 34°11.081' E 141°36.204'
812	Upstream of Renmark at **Murtho**	S 34°04.106' E 140°48.668'
814	Downstream of Murray Bridge at **Woods Point**	S 35°13.966' E 139°24.895'

Carbon:Nitrogen (C:N) ratio

The Centre for Freshwater Ecosystems (CFE) has many databases that have been collected as part of food web studies. We re-interrogated these databases as they also include the carbon and nitrogen ratios of numerous macroinvertebrates and fish that have been collected throughout the Murray–Darling Basin (MDB). Sites included, the Murray, Ovens, Murrumbidgee and Condamine Rivers and upland streams near Falls Creek. Taxa were aggregated as appropriate (generally family for macroinvertebrates and species for fish), giving a compilation of the C:N ratio of the macroinvertebrates (prey) and fish tissue (consumers).

Macroinvertebrate biomass

The biomass of Murray River macroinvertebrates were derived from a range of sources. In some instances, biomass of common taxa were obtained from published descriptions. Where data were not available, published length-to-mass ratios were used to infer biomass of given taxa (Stoffels *et al.* 2003). For the latter, a combination of published measurements, as well as measurements made directly on specimens collected from the Murray River were combined to generate a database on the average mass of individual and combined taxa. Biomass was standardised to the sample units used in the RMBMP.

Data modelling

For the first step in the analysis, we prepared general visualisations to determine whether there were any obvious trends in the biological components and flow over time. We then used these data in a Generalised Additive Modelling approach to determine relationships between biomass and flow.

We examined whether the following 12 environmental predictor variables could explain the patterns in prey biomass and community structure:

- Mean maximum air temperature.

- Mean log-normalised discharge (log-normalised so that sites with different mean discharges could be modelled as one).

- Standard deviation of flow.

Each of the variables were calculated over 3 months, 6 months, 1 year and 2 years prior to sample collection date; thus resulting in four time periods for each parameter. The four time periods of each parameter led to the 12 predictor variables in total.

Results and Discussion

Community composition

Long-term monitoring showed that the macroinvertebrate communities at sites along the Murray River have changed over time (Figure 1). The trajectories in figures 1A, B and C gave a visual interpretation of how the community composition trajectories at each site compared with one another. When the community composition for all sites were examined collectively, the upper (sites 801 and 804) and lower (site 814) sites showed the greatest change in community composition (Figure 1D). While some change occurred at Lock 9 and Euston, the change was not as dramatic as at the other sites. Interrogation of the data suggests that some of the key taxa contributing to the greatest dissimilatory among sample times and sites were mayflies (Caenidae), caddisflies (Ecnomidae) and worms.

C:N ratios

Interrogation of several CFE data sets provided C:N ratios for 1155 specimens, including macroinvertebrates and fish, representing 132 unique taxa and 93 families. These data were aggregated to appropriate taxonomic relevance, which yielded C:N ratios for 90 taxa; data includes 18 fish species (Figure 2). Limited variability occurred in the C:N ratios of some groups (e.g. shrimp and prawns), reflected by the narrow boxes in the box plots. By contrast, there was a wide range in the C:N ratios of other groups, such as worms. This most likely represented the ratio of particular species that once aggregated led to high variability at the family level.

Notably, all of the fish species in our data set had reasonably similar C:N ratios, ranging from approximately 3.5 to 4. Fish C:N ratios seems to be similar to other freshwater fish. For example, multiple populations of a single species (white fish – *Coregonus clupeaformis*), sampled across the great lakes of USA had C:N ratios between 3.45 and 3.97 (Fagan *et al.* 2011). C:N ratios can vary, often depending on the growth conditions of individuals. Broadly speaking, ratios greater than 3.5 generally reflect increased lipid content of animals, or particular tissues (Nogués *et al.* 2008), but the relationship between ratio and lipid content is not necessarily simple (Fagan *et al.* 2011). It is possible that our specimens with ratios closer to 4 may have had higher lipid content.

Crustacean taxa (prawn and shrimp species) are known to be important food sources for fish. Examination of their C:N ratios suggested that the freshwater crustaceans (prawns - (*Macrobrachium austrlaiense*/shrimp – *Paratya* spp.) had C:N ratios most similar to those of fish species. Other macroinvertebrates generally had ratios considerably higher than fish, but there was considerable variation within families. For example, although there was strong variability amongst the chironomids (Figure 2#2) and worms (Figure 2#4), both had ratios considerably higher than fish. Based on stoichiometric theory, the carbon and nitrogen imbalance will mean that consumers will have to consume a greater amount of their prey to obtain the same level of nitrogen in their diets. This would result in a greater expense of energy, or an energetic cost to the consumer.

Initial examination the C:N ratio over time indicated that overall, the mid sections of the river showed no response over time (Figure 3). There was considerable variability at the upper Murray sites (Biggera and Jingellic), which coincide with the sites with greatest diversity.
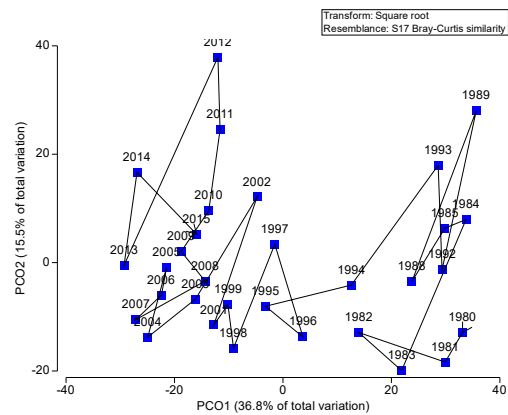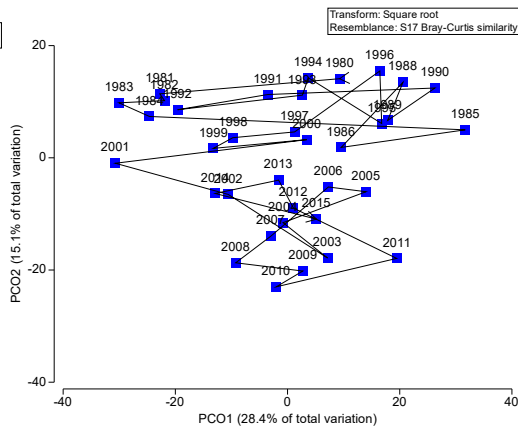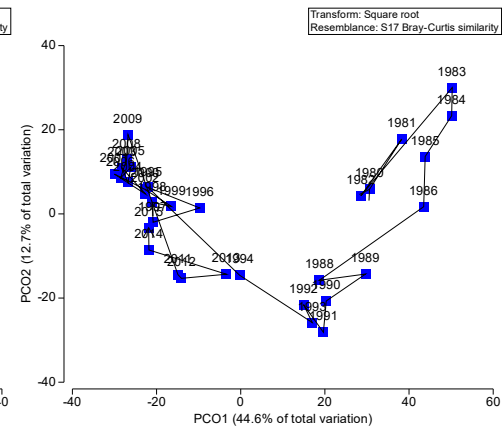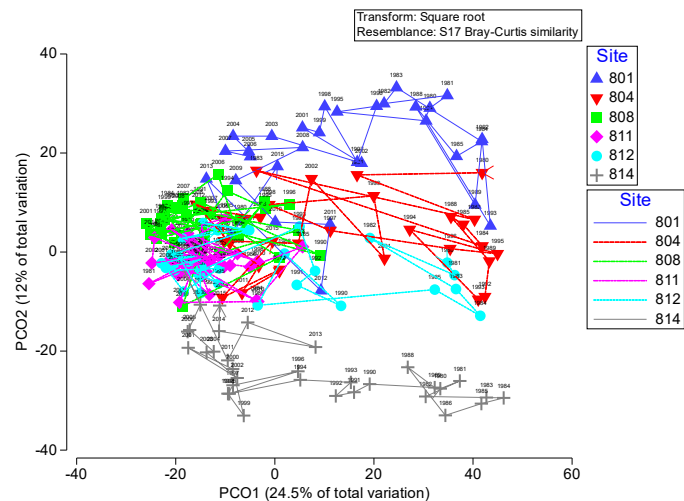
Figure 1. Macroinvertebrate community composition at representative sites (symbols) on the Murray River, with respective trajectory (line) over time. All data were measured as part of the Murray River Monitoring Program, from 1980 to 2015. Individual sites include Jingellic (A), Euston (B), Woods Point (C), with all sites and times combined in panel D.
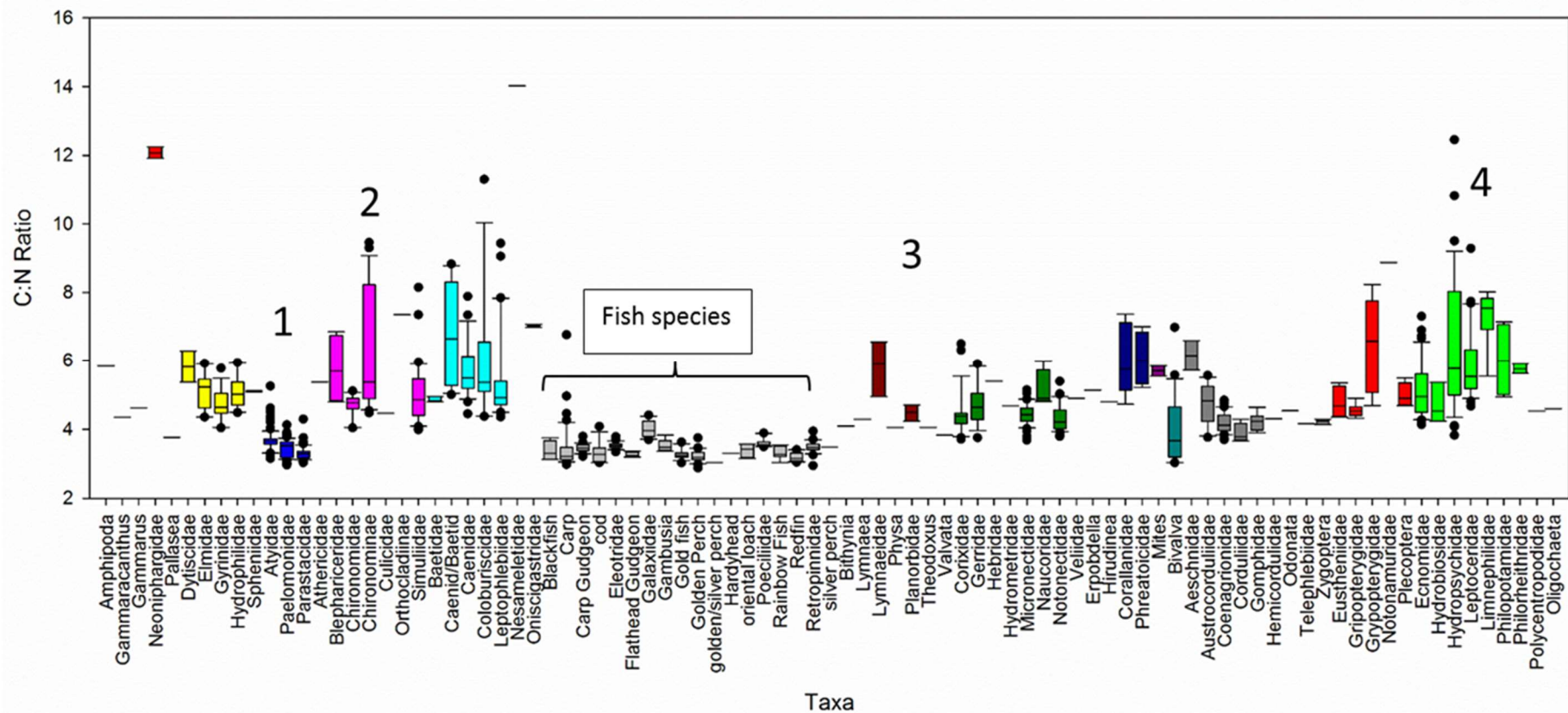
Figure 2. C:N ratios of a wide range of aquatic taxa in the Murray–Darling Basin. Most macroinvertebrates have been aggregated to family, with fish presented as species. Relevant taxa that have been highlighted: 1) shrimp and prawns, 2) chironomids, *Physa* (snail), 4) worms and fish.
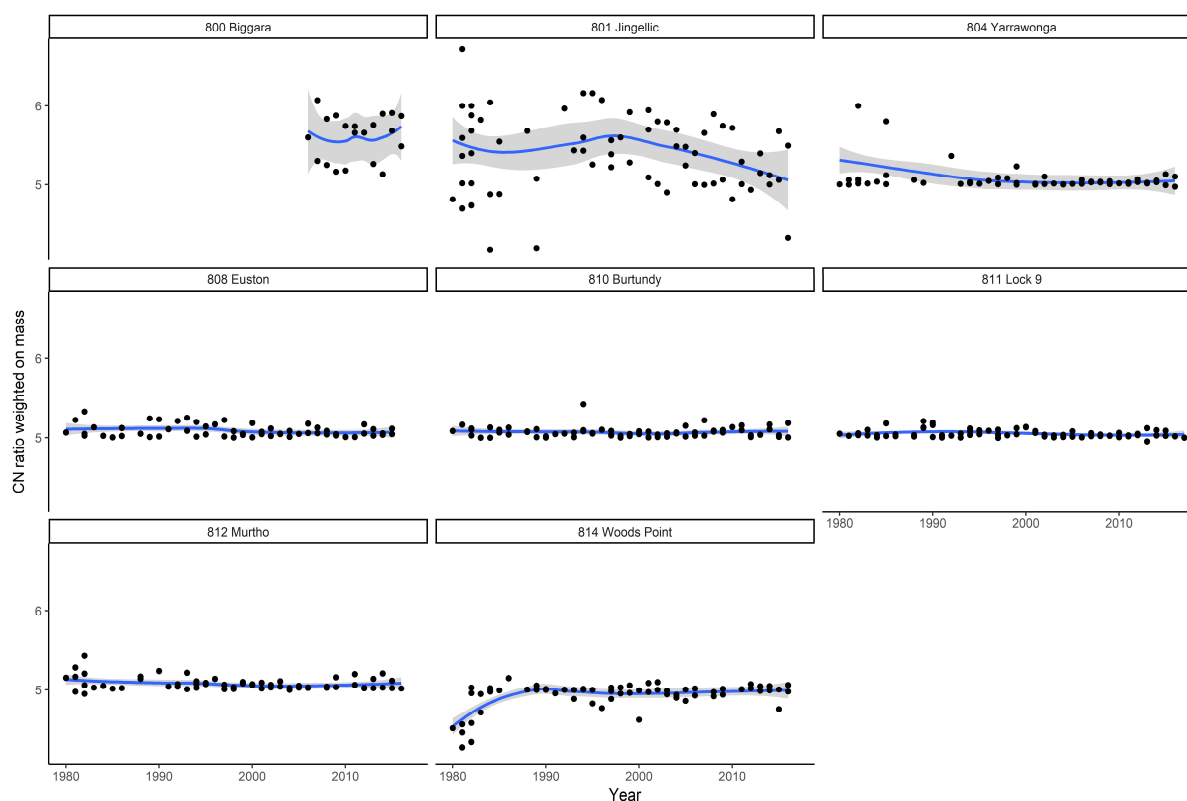
Figure 3. C:N ratio of the benthic macroinvertebrates over time.

Modelling

Simple visual analysis of the macroinvertebrate biomass (Figure 4) middle row) broadly showed a similar pattern across the three contrasting sites on the Murray. The peak in biomass appeared to coincide with the major shift in flow. Similarly, species richness (bottom row) showed a similar reproducible pattern among sites over time, generally with low richness in the 1980s, a steady increase through the 2000s, and then a decline in the late 2010s. There were some subtle differences; for example, a much broader maximum occurred at Jingellic, whereas Lock 9 had a narrow peak in richness. A general observation suggests that the species richness was not following a clear association with particular flows. Modelling supported the visual analysis in suggesting that the variables used here could not be used to predict species richness. Similarly, the variables used here could not predict the C:N ratio landscape.

Of the 12 predictor variable noted above (3, 6, 12 and 24 months each for mean maximum air temperature, mean log-normalised discharge and standard deviation of flow), normalised discharge 6 months and 1 year prior to sampling provided the best fit for the model; however, the predictor variables explained less than 8% of the variance. None of the time periods for mean maximum air temperature and standard deviation of flow showed a relationship with biomass.
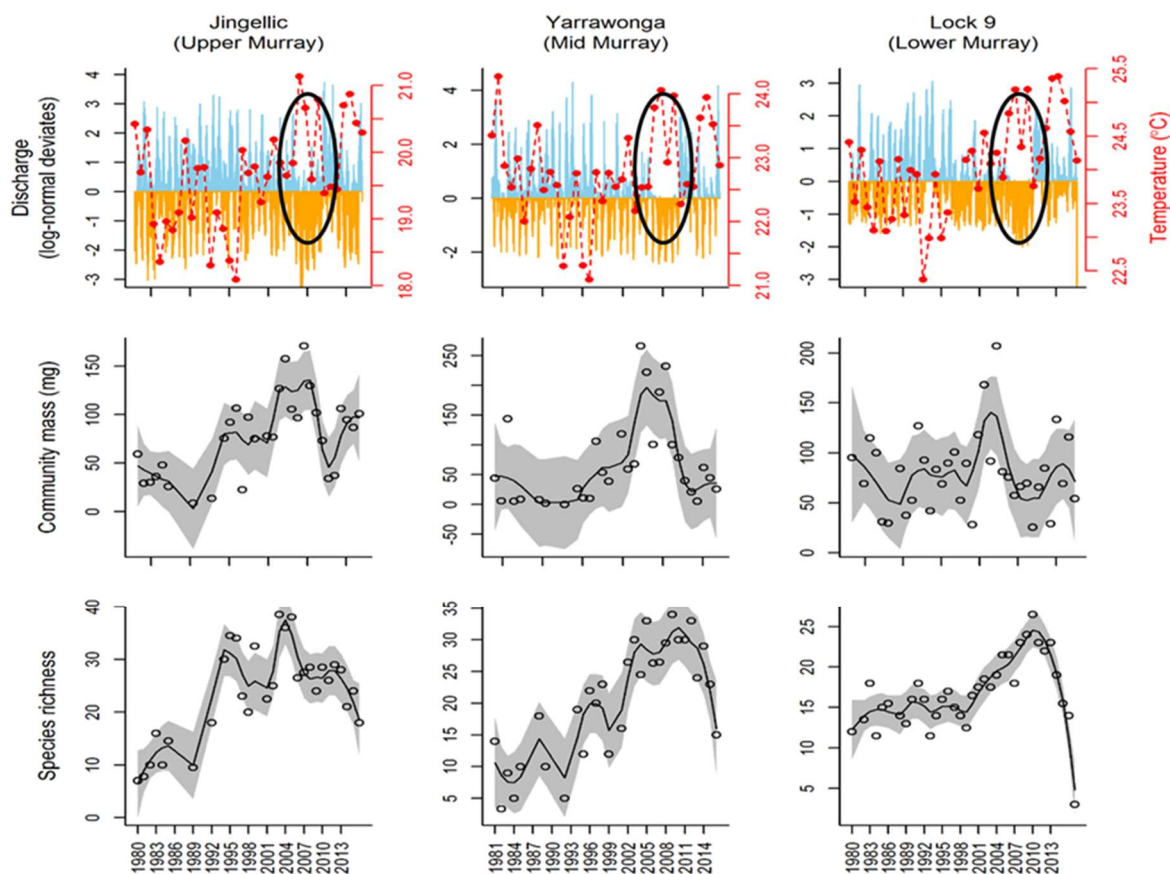
Figure 4. The long-term response of benthic macroinvertebrate biomass and species richness to discharge. The top row shows average flows from 1980 to 2013. The blue line shows when flow was above average, and the orange line shows when flows were below average, with ovals highlighting the millennium drought. The middle row shows community biomass response over time and the bottom row shows species richness over time.

Summary

Previous work examining the entire macroinvertebrate RMBMP data set showed that macroinvertebrate taxa richness and abundance increased across all sites after the 1993 flood and declined after peaking during the millennium drought (Paul *et al.* 2018). In their approach, environmental variable (nutrients, salinity, and water temperature) only provided a partial explanation of the community patterns.

Our analysis, which was based on quantitative measurement of the benthic invertebrates, suggested a similar response in abundance, but the richness was considerably more variable with respect to sites. Our emphasis was to investigate whether a range of flow parameters could explain macroinvertebrate abundance. If this occurred, then it would be possible to design environmental flows that wold generate predictable outcome in terms of macroinvertebrate biomass, or in other words, energy available to higher organisms. Our modelling presented here showed flow no relationship to the overall nutritional value (measured as C:N ratio), or species richness, and of the flow variables could only explain a very small part of the variation in macroinvertebrate biomass. Several reasons may contribute to this observations, but it is likely that flow on its own does not represent a single limiting factor, and that a combination of factors are driving the biomass. By way of example, while optimal flow characteristics may be developed, these will still fall short in producing appropriate biomass if there is insufficient surface material (such as snags) to allow for proliferation of macroinvertebrates.

Decapod nutrition

Background

Decapods (yabbies, shrimp and prawns) are primary consumers in Australian river systems, eating an array of food sources, from plant and animal detritus to biofilms on solid surfaces. Since decapods, like yabbies, can consume diverse food sources, there is considerable opportunity for them to consume foods of varied nutritional quality. Furthermore, since decapods themselves are important prey for native fish, representing a key step in the riverine food web, reduced nutritional quality of the primary resources could manifest itself through the food web to higher consumers such as fish.

At the outset of this project, nutritional literature recognised that amino acids and fatty acids could potentially be important regulators of growth of consumers and that there was evidence demonstrating the importance of high quality food sources in stream food webs (Guo *et al.* 2016). At the biochemical level, the aquaculture industry has identified a suite of 'indispensable amino acid requirements (IAA)' (also termed essential amino acids) for fish growth (Cowey 1995; Mambrini & Kaushik 1995) and sources of protein to regulate amino acid composition in eggs (Gunasekera *et al.* 1996).The amino acid composition of trout cod and Murray cod eggs and larvae have been examined to better understand survival during the early development stages (Gunasekera *et al.* 1999). Studies also suggest different pools of amino acids can play a metabolic role, can be assimilated for growth, but also other biochemical functions such as osmotic regulation (Gunasekera *et al.* 1999). While the aquaculture industry developed understanding on amino acid requirements, there was little or no available information on how this might translate to real world scenarios and food webs. Thus the fundamental aspects of amino acids as resources for consumers was explored as part of a PhD project (see Shakaya et al 2019, MMCP Education report)

Higher organisms have a dietary requirement for poly unsaturated fatty acids (PUFA) and since some PUFA cannot be synthesised by consumers in sufficient quantity to meet basic biologicals demands, these molecules have been termed 'essential PUFA' and must be obtained from consumer diets., Of the essential PUFA that exist in cells, four are of particular interest, namely eicosapentaenoic acid (EPA), a-linolenic acid (ALA), docosahexanoic acid (DHA) and arachidonic acid (ARA). While aspects of the biochemical requirements of PUFA are developing, studies show that these essential PUFA are critical for development, reproduction and hormone regulation in many animals (Guo *et al.* 2016).

Essential PUFA are primarily synthesised by autotrophs and green algae in particular is known to contain the highest concentration of these molecules among basal food resources. Managed flows can lead to floodplain connection and in doing so, provide possible connection for biota to pass between river channels and floodplains. The river-floodplain connection will provide a diverse range of food resources (and potentially higher concentrations of high-quality algae) and in doing so, is likely to enhance the overall nutritional value of the resources, leading to prey for fish of higher nutritional value.

The decapod nutrition component of the project involved two activities: 1) examining growth rates of yabbies under laboratory conditions, being fed diets of different quality, and 2) a field program that examined the nutritional value of yabbies collected from riverine and floodplain environments. The activities were addressed by:

1. Determining growth rates on diets that were either detritus - (high plant content and poor quality) or chironomid-based (high protein content and good quality).

2. Analysing the nutritional value of yabbies growing on the experimental diets.

3. Contrasting the nutritional value of yabbies from river channels and their associated floodplains.

Laboratory trials

Methods

Experimental set up and design

Three different dietary scenarios were tested on three different sets of yabbies. One group received a good quality diet of bloodworms (chironomids of the sub family Chironominae), containing high protein that is purely animal based. The second group received a diet of commercial freshwater crayfish pellets, as an intermediate or control diet, that was plant and animal balanced. The third group received a poor-quality diet comprised of leaf detritus that was low in protein and purely plant based.

Unfiltered water from the Murray River was used in the tanks, and zooplankton had not been removed. Each yabby was separated into a labelled 1 L plastic food storage container that had holes for water circulation. Rocks were place on the top of each container in the tank to secure it in place (Figure 5).
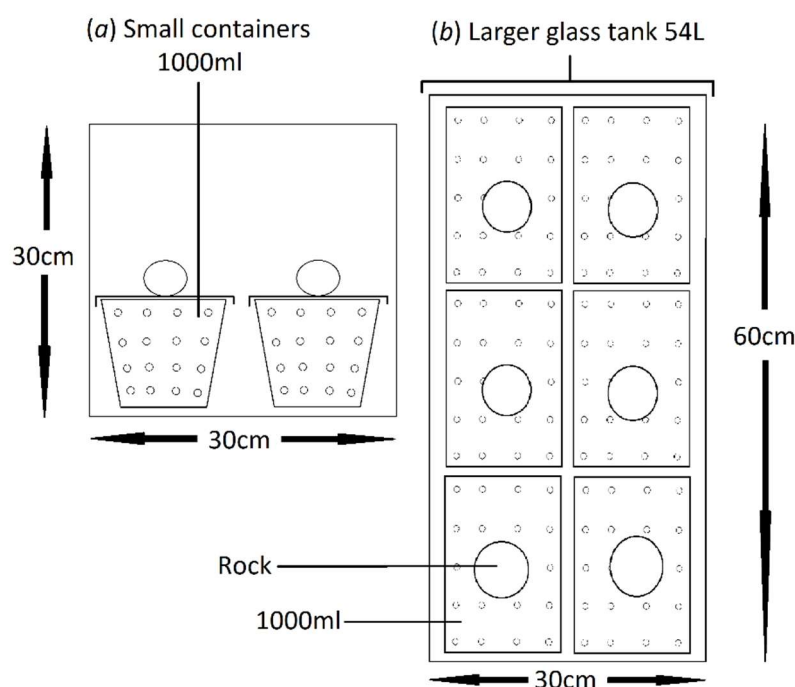
Figure 5. The tank system showing the arrangement of small 1000 ml holding containers (a) within the larger glass tank (b).

There were six tanks per row (except for sump system B), and one row per sump system (apart from sump system C, where there were two). Groups were divided vertically across all three systems, with 40 yabbies in each dietary group (Figure 6).
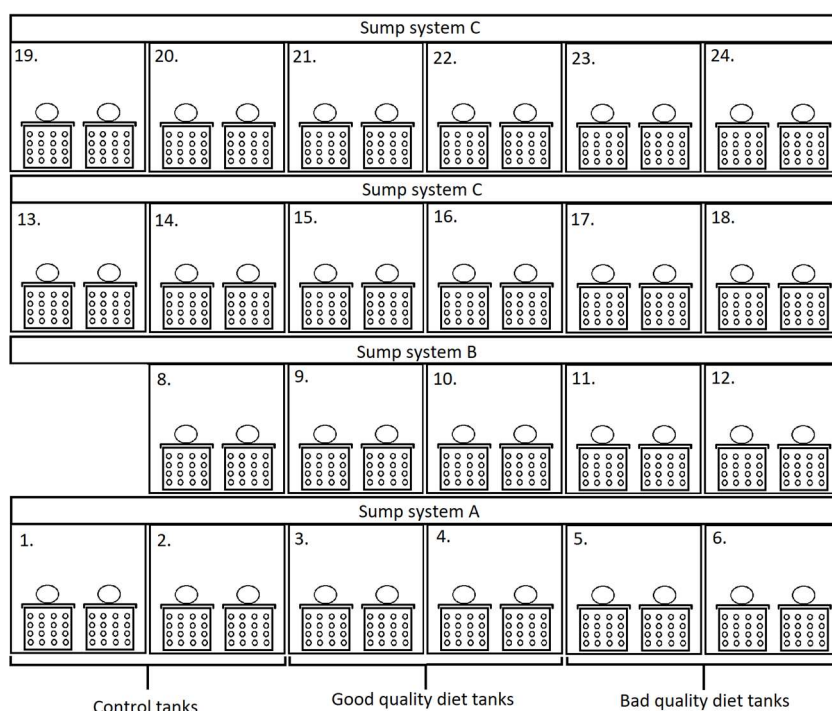
Figure 6. Dietary grouping across tank systems.

Yabbies were fed 0.08 g of either frozen bloodworms, detritus, or freshwater crayfish pellets once a week for 60 days. The bloodworms and freshwater crayfish pellets were both bought commercially; however, the detritus was made into agar set pellets. The pellets were made from 40 g of aged/soaked river red gum leaves, 100 ml of water and 1.5 g of agar. First, 20 g of leaves were added with 100 ml of water, blended until semi-smooth, and then the remaining 20 g of leaves were added and blended until smooth and thick. Agar was added and heated in a microwave for 30 seconds at a time to dissolve the agar. The mixture was set in small trays and once set was cut into 0.08 g cubes. The pellets were kept in a freezer until use.

On the day of feeding, the yabbies were initially placed on paper towel for 5 seconds to remove excess water and then weights were measured and recorded. The date, time, tank number, sump system, container number, pigmentation and mortalities were noted. The food was then placed in the container, the lid was placed back on and the yabbies were returned to the tanks. The amount of food given per tank was recorded, and the following week the amount left over was recorded and used to form a percentage of how much was being consumed per tank.

The tanks were cleaned weekly to remove any excess food and faeces and topped up with river water. A YSI Pro DSS water quality meter was used to measure water quality daily for temperature (°C), pressure (mmHg), dissolved oxygen (DO% and mg.L^{-1}), conductivity (SPC-μs.cm^{-1}), pH, pH mv and turbidity (NTU).

Chemical analyses

After their final weighing, 10 yabbies from each treatment were frozen. Each yabby was blended in a known volume of water and re-frozen. A proportion of the sample was freeze dried and the C:N ratio determined by LECO analysis (Southern Cross University).

C:N ratio analysis

The remaining homogenised samples were freeze dried and the powder analyses for % carbon and nitrogen were determined by LECO analysis at the Environmental Analysis Laboratory, Southern Cross University.

Data analysis

Weights on individual yabbies were recorded and converted to a percent change based on individual mass. This approach allowed us to combine data from yabbies with contrasting starting weights. We used analysis of variance to compare response to treatments. Data were log-transformed as necessary to ensure homogeneity of variance.

Results and Discussion

Yabby growth

Growth on the detritus alone was the slowest of the three food sources (Figure 7). Growth curves were best modelled by linear kinetics (r^2 0.822), with a slope of slope of 0.115/d. Bloodworms also linear fit (r^2 0.965), but with a slope of 0.433/d. Growth on the positive control (commercial pellets) was the fastest of the three food sources and followed a three parameter first order growth (r^2 = 0.986). These results showed that the both bloodworms and detritus in particular, while supported growth, were limited in some respects. Exploring all of the possible micronutrient deficiencies was beyond the scope of this project, but we have demonstrated that major changes in the diet can lead to remarkable changes in the growth rates of yabbies.
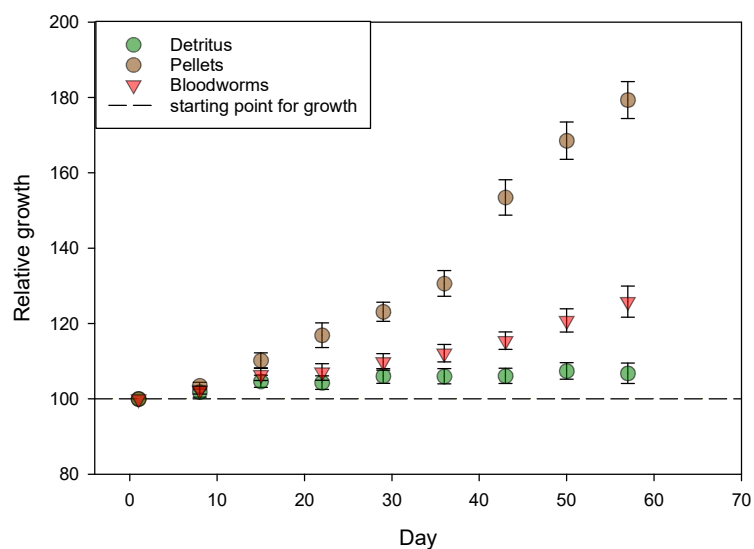
Figure 7. Growth of yabbies on detritus (green circles), bloodworms (red triangles) and commercial pellets (brown circles).

C:N ratio

The C:N ratios of the bloodworm, detritus and pellets used as the food source were 4.6, 38 and 6.8 respectively. The average (±SD) C:N ratios of yabbies grown on bloodworms, detritus and pellets were 4.1 (0.1), 4.4 (0.3) and 4.6 (0.1), respectively (Figure 8). Small but significant differences were measured in the C:N ratios of yabbies grown on the different treatments (ANOVA, $p < 0.05$ in all cases).
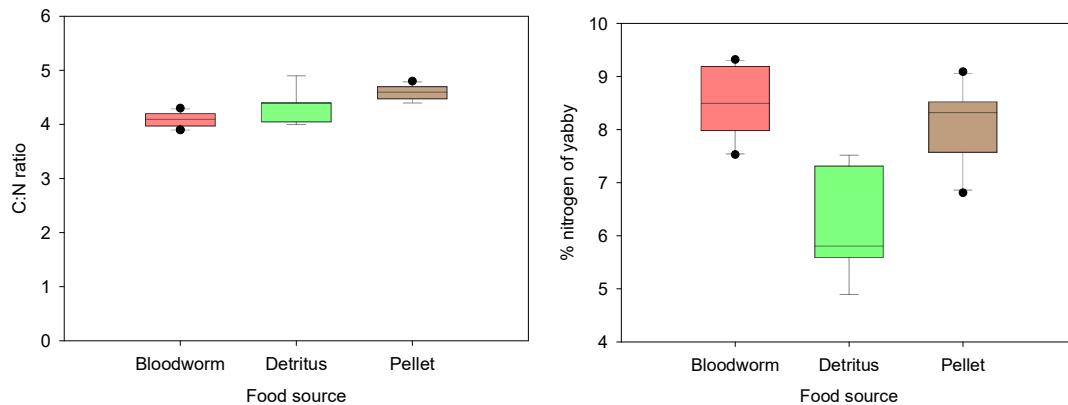
Figure 8. Box and whisker plots showing C:N ratios (left panel) and % nitrogen in yabbies fed bloodworms, detritus and commercial pellets. The box shows 25 and 75% percentiles with the line showing the means. Bars are the 10 and 90% percentiles.

Percentage Nitrogen in biomass

The average (±SD) percentage nitrogen (%N) of yabbies grown on bloodworms, detritus and pellets were 8.5 (0.6), 6.2 (0.9) and 8.1 (0.7), respectively, with the yabbies growing on a detritus diet having a significantly lower total %N in their biomass than those subjected to the other two treatments (Figure 8). While yabbies can grow on detritus, these data suggest that factors which lead to a diverse diet produce yabbies that are a 'more nutritious prey'.

Summary

Diet had a profound effect on the growth of yabbies. As we expected, a detrital diet alone, with a very high C:N ratio provided nourishment for yabbies, but they barely increased their body mass over the entire experiment. Significantly faster growth occurred on bloodworms, but not as great as on a commercial pellets. This shows that C:N ratio was not an ideal predictor. Commercial pellets contain a range of macro and micronutrients and while bloodworms do allow for growth to occur, other nutrients become limiting. Detritus-fed yabbies also had the lowest proportion of N in their biomass; there was no difference between blood worms and pellets.

Taking these data in combination, yabbies require a diverse range of food sources to maintain highest growth rates. We are not aware of data on the growth rates that are actually achieved in nature and it is quite reasonable to think that with the additional expense of energy required to gather food (as compared to laboratory trials), the rates we achieved are possibly inflated, relative to those in nature. Be that as it may, the ability to seek out diverse food sources would be seen as an advantage and thus the eflows that provide contact with floodplains would likely lead to yabbies that are able to take advantage of different food sources.

Field study

Methods

Site and sampling description

Eight river and wetland sites were selected from the lower Ovens River floodplain (Figure 9). Each river site was defined as a 200 m reach and permanently-filled billabongs were chosen as the wetland sites.

Figure 9. Typical wetland (left) and river channel (right) sites where yabby nets were deployed.

Three nets were placed on the banks of the river and edge of each wetland site, just below the water level, and retrieved approximately 24 hours after deployment. Animals in each net were counted and the first 10 specimens were collected for nutritional analysis and placed in a freezer upon retrieval. Water samples were also taken for total nitrogen, total phosphorus, dissolved nutrients, dissolved organic carbon and chlorophyll-*a*.

Samples were pre-treated for fatty acid analysis to compare gut contents (diet) and whole animal (nutrition as prey). On return to the laboratory, animals were thawed and the gut contents removed from specimens. Whole animal samples and gut contents were homogenised and quickly refrozen before dispatch for fatty acid analysis.

In all, three field trips were carried out to collect a statistically representative number of specimens.

Fatty acid analysis

A known volume of homogenised sample was stored frozen and sent to Deakin University for fatty acid analysis. Fatty acid analysis followed those methods described previously (Conlan *et al.* 2017). Briefly, for all samples, lipid was extracted from dry samples soaked in dichloromethane:methanol (CH_2Cl_2:CH_3OH) and quantified gravimetrically on a four-figure balance. Lipid class analysis used an Iatroscan MK 6 s thin layer chromatography-flame ionisation detector. Fatty acids were then extracted and esterified into methyl esters using the acid catalysed methylation method (Christie 2003). Gas chromatography was then used to identify the fatty acid methyl esters relative to known external standards. Four key essential fatty acids, namely eicosappentaeonic acid (EPA), docosahexaenoic acid (DHA), arachidonic acid (ARA) and alpha-linoleic acid (ALA) were the focus of the analyses as they represent four of the key fatty acids that are required in a diet of fish.

Chemical analysis

Dissolved organic carbon (DOC), chlorophyll-*a*, total nitrogen, total phosphorus, and dissolved inorganic nitrogen were all measured by standard methods, to National standards of quality control and quality assurance, in the CSIRO Analytical Chemistry Laboratory (Wodonga).

Total fat content was measured by mass following organic solvent extraction of yabby tissue.

C:N ratio was determined as described above.

Results and Discussion

Water quality

DOC concentrations were ranged between 2–3 mg.L^{-1} in the river channel, which is typical of the lower Ovens River (Hadwen *et al.* 2010) (Figure 10). DOC was highly variable amongst wetlands, with those smaller wetlands possessing large amounts of overhanging red gum trees and litter generally having the highest levels of DOC. The water column chlorophyll-*a* concentrations were also typical of wetlands, demonstrating extensive levels of algal production within the water column.
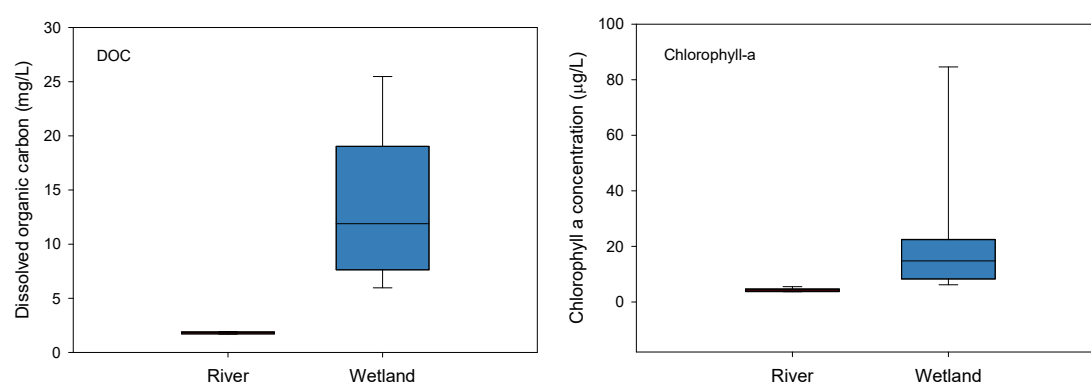
Figure 10. Dissolved organic carbon (left) and chlorophyll-*a* (right) in the Ovens River and wetland samples.

High levels of production in the wetlands were also demonstrated by the high level of total nitrogen and phosphorus within the wetlands. Again, these levels were quite variable amongst the wetlands and similarly a likely reflection of their size and place on the floodplain. River channel concentrations were also typical of the lower Ovens River. Very low oxides of nitrogen (NO$_x$) levels across wetlands is consistent with anaerobic conditions providing the necessary environment for denitrification to occur (Figure 11). The ammonium concentrations were not remarkably different between the river channel and the wetlands (Figure 11).
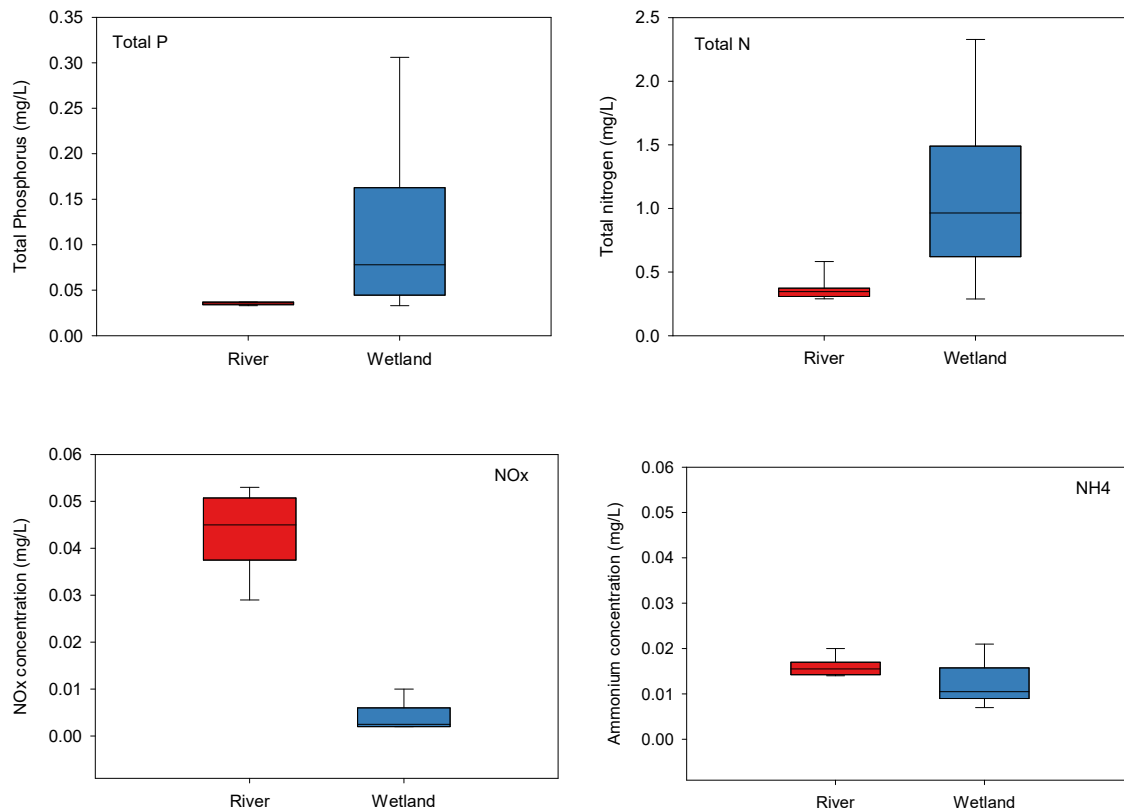
Figure 11. Total phosphorus (top left), total nitrogen (top right), oxides of nitrogen (bottom left) and ammonium concentrations (bottom right) in wetlands and the river channel of the Ovens River floodplain.

Yabby C:N ratio

The average (standard deviation) of C:N ratio of yabbies collected from wetland and river sites were 4.2 (0.2) and 4.3 (0.1), respectively, demonstrating that there was no significant difference in the C:N ratio of yabbies from different sites.

Total fats

The average (standard deviation) of total fat content of yabbies collected from wetland and river sites were 1.22 % (0.54) and 0.94 % (0.74), respectively, demonstrating that there was no significant difference in the total fat content of yabbies from different sites.

Fatty acid analysis

The fatty acid composition in the guts of yabbies was significantly different from that of the yabbies themselves Figure 12. The spread among the individuals indicates that the diets were more variable than the animals themselves.

Figure 12. Nonmetric multi-dimensional scaling ordination depicting the fatty acid composition of yabby diets (blue triangle) and the fatty acid composition of the animals themselves (red triangle)

Total PUFA amongst individuals collected from wetlands, with the 10 and 90% percentiles ranging between 20 and 40%, was marginally different from those in rivers (Figure 13). T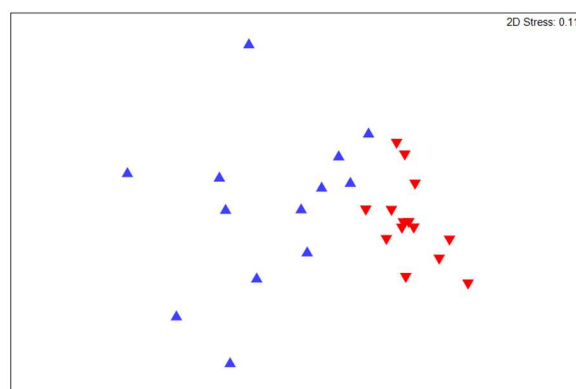he differences in the mean values among the yabbies from wetlands and rives were not great enough to exclude the possibility that the difference was due to random sampling variability; i.e. there is not a statistically significant difference (ANOVA, P = 0.107).
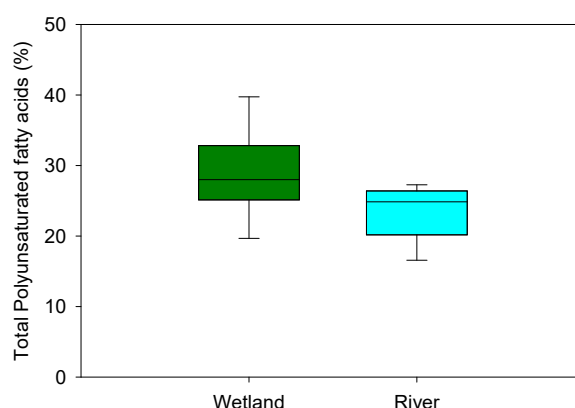

Figure 13. Contribution of unsaturated fatty acids present in yabbies from wetlands and rivers

Each of the four fatty essential fatty acids present in yabbies demonstrated important differences between those present in wetlands and rivers (Figure 14) Yabbies from wetlands were significantly more enriched in ALA than those in the river channel. Site of origin played no role with respect to DHA in the yabbies. Riverine yabbies were generally more enriched in EPA than those in wetlands, although there was a large variation in the concentration among the riverine yabbies. Similarly, ARA was not greatly different in yabbies from the river and wetlands, but again, there was a large degree of variation in the amount of ARA in riverine yabbies.

The high level of ALA in wetlands is consistent with previous reports that show wetland seston is more enriched than riverine seston (McInerney *et al.* in prep), suggesting that seston was the primary source of ALA. This reflects the increased concentration of certain algae known to synthesise ALA. In contrast, while previous reports show wetland seston was enriched in EPA, this was not reflected in yabbies. In this situation, riverine yabbies are gaining their EPA from an alternative source. The likely source is algal communities present on biofilms, as opposed to the seston. Previous work shows that ARA typically constitute between 0.5 and 2.5% of the fatty acid content of wetland seston, and only between 0.01 and 0.04% of the fatty acids in rivers. ARA is considerably more enriched in yabbies in wetlands, averaging more than 8% of the fatty acids. These

yabbies represent a very good source of this essential fatty acid. While some riverine yabbies had this level of ARA, it was not consistent among animals. This again is a likely consequence of riverine seston being low in ARA, but some yabbies are able to obtain large amounts from biofilm algal sources.
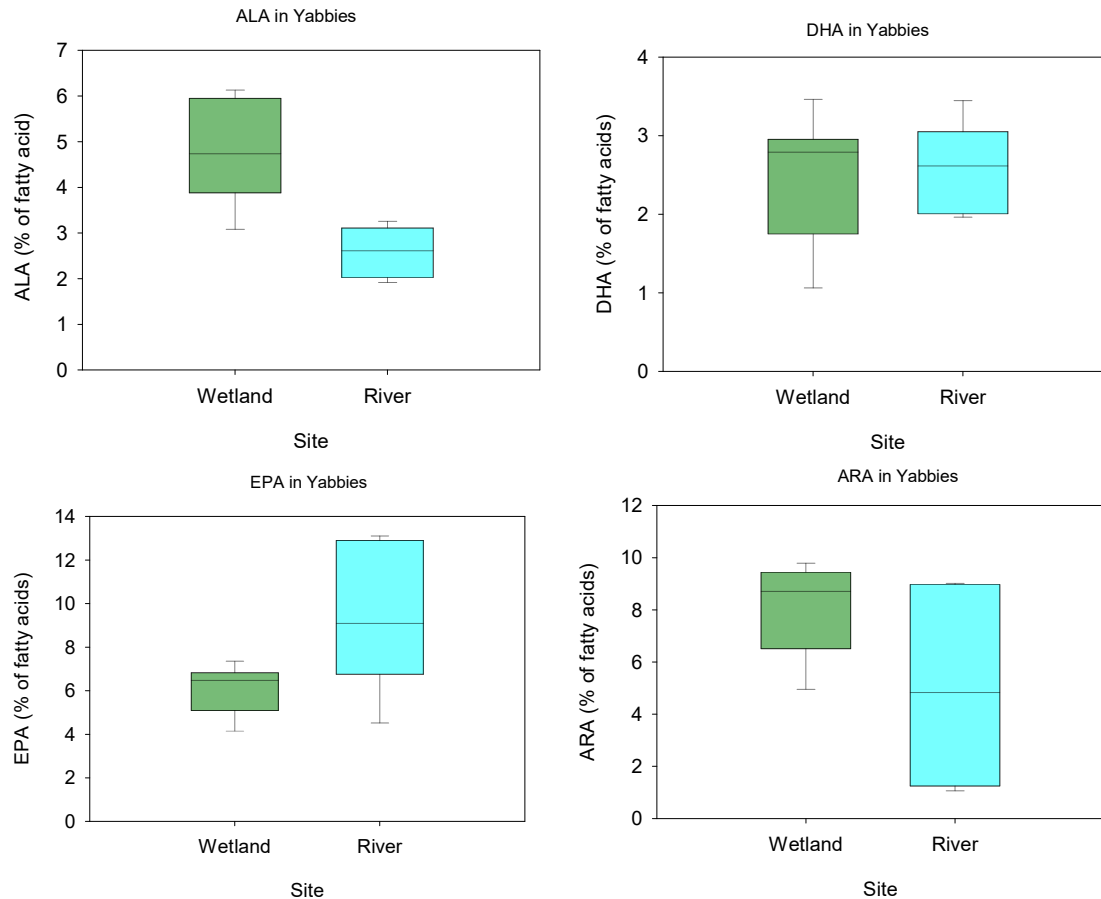
Figure 14. Four essential fatty acids present in yabbies collected from wetlands and the river channel of the Ovens River floodplain: ALA (top left), DHA, (top right), EPA (bottom left) and ARA (bottom right).

Summary

The nutritional quality of food resources has emerged as an important consideration when examining factors that drive food web structures. Essential fatty acids are required in the diet of animals as they are unable to be synthesised by the animals themselves. In this study, we examined how the nutritional value of yabbies changed in response to two environments; namely wetlands and rivers. By examining the levels of four essential fatty acids in yabbies from the two environments, we confirmed that the necessary food resources were available in rivers and wetlands alone, but that rivers and wetlands each provided a specific enriched source of one or more fatty acids.

Taking this information, management actions that allow transfer of yabbies between wetlands and rivers would provide the best nutritional landscape for consumers of yabbies (notably fish).

Management implications

This component of the MMCP had three overarching research questions:

Can flow variables be used to predict the quantity and quality of food resources in rivers?

Do primary food resources alter the growth rates and nutritional quality of key fish prey?

Does floodplain connectivity lead to any improved nutritional quality of key fish prey?

Macroinvertebrate community response to flow

Our modelling, based on a quantitative measure of the benthic invertebrates showed that there was no relationship between flow and the overall nutritional value (measured as C:N ratio) or species richness. The flow variables we examined could only explain a very small part of the variation in macroinvertebrate biomass (effectively amount of energy available to consumers). These results suggest that a combination of factors are driving the biomass, with other limiting factors playing a clearer role in driving abundance.

Taken in isolation, macroinvertebrate abundance (measured at the scale of this component) can not necessarily be predicted for a series of flow variables and should be considered in combination with other factors that are likely to promote increased abundance, e.g. woody debris. Measuring food availability (and quality, see below) needs to be a component of complimentary measure implementation (e.g. addition of woody debris), and carried out in conjunction with flow modifications.

Yabby nutritional ecology

We showed that yabbies require a diverse range of food sources to maintain highest growth rates and that diets resulted in a small but significant change in their nutritional value as prey for fish.

Furthermore, our field studies showed that while essential fatty acids could be derived from their food sources in rivers and wetlands, as each site differ in its ability to supply the essential fatty acids. Thus, an optimum balance of essential fatty acids would be achieved for yabbies if they were presented with both wetland and river conditions.

Here we provide empirical evidence that can be used to provide evidence based information on the value of environmental flows. We also provide a valuable basis for modifying any continued monitoring, particularly with the development of complimentary measures in river system management.

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