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To cite this article: Ivor Gowns, Darren Ryder, Paul McInerney, Nick Bond, Galen Holt, Rebecca Lester & Ross Thompson (2020) The use of fatty acids to identify food sources of secondary consumers in wetland mesocosms, Journal of Freshwater Ecology, 35:1, 173-189, DOI: [10.1080/02705060.2020.1761463](https://doi.org/10.1080/02705060.2020.1761463)

To link to this article: <https://doi.org/10.1080/02705060.2020.1761463>



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The use of fatty acids to identify food sources of secondary consumers in wetland mesocosms

Ivor Grown^a, Darren Ryder^a, Paul McInerney^b, Nick Bond^b, Galen Holt^c,
Rebecca Lester^c and Ross Thompson^d

^aSchool of Environmental and Rural Science, University of New England, Armidale, NSW, Australia;

^bCentre for Freshwater Ecosystems, La Trobe University, Wodonga, VIC, Australia; ^cCentre for Regional & Rural Futures, Deakin University, Geelong, VIC, Australia; ^dInstitute of Applied Ecology, University of Canberra, Canberra, ACT, Australia

ABSTRACT

Increasing demand for freshwater during the last century has so severely degraded many wetland ecosystems that they are some of the most seriously impacted environments in the world. Environmental flows are used as a management tool to restore parts of the hydrological regime altered by human water use, to rehabilitate these wetlands. Research and monitoring to date has focused on understanding 'flow-ecology' relationships, without investigating the mechanisms underlying them. We sought to understand how different basal food sources are incorporated into fish tissue in temporary wetland systems. This study provides a necessary first step toward the development of mechanistic research that investigates the effects of variation in fatty acids (FA) within the food and prey base on top predators. We added different sources of fatty acids to wetland mesocosms by adding extra food sources including redgum leachate to increase planktonic bacteria populations, cyanobacteria, green algae and biofilm matrix to observe how they were incorporated into secondary consumers. Wetland soil and water was added to replicate mesocosms, left for 28 days to produce zooplankton and then Western carp gudgeons added. There was a clear shift in the invertebrate assemblage structure following the introduction of the gudgeons. There was also a clear difference in assemblage structure and nutritional value between benthic and planktonic invertebrates. However, the addition of extra food sources did not generate differing FA profiles between treatments in the substratum fractions, invertebrates or fish after 14 days. We suggest that food sources generated within the mesocosms themselves may have outweighed any treatment effects. Using flooded wetland mesocosms potentially would have provided more realistic knowledge of the food web mechanisms of wetland inundation rather than feeding zooplankton fed specific primary food sources to fish. However, future experiments attempting to identify the mechanisms of the transfer of basal food sources in wetlands to secondary consumers may wish to directly feed fish primary consumers raised on specific basal food sources.

ARTICLE HISTORY

Received 13 November 2019

Accepted 20 April 2020

KEYWORDS

Hypseleotris; fatty acid;
food web; mesocosm;
environmental water

CONTACT Ivor Grown  igrown@une.edu.au  School of Environmental and Rural Science, University of New England, Armidale, NSW 2350, Australia

 Supplemental data for this article is available online at <https://doi.org/10.1080/02705060.2020.1761463>.

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Introduction

Increasing demand for freshwater during the last century has severely affected wetland ecosystems to the point that they represent some of the most seriously degraded environments in the world (Lemly et al. 2000; Vorosmarty et al. 2010; Davidson et al. 2018). Most the world's river systems are now subject to human impacts (Tonkin et al. 2019), requiring increasingly sophisticated approaches to river management (Thompson et al. 2019). An important tool which has emerged has been the management of flows to restore, protect or enhance environmental outcomes both in-channel and in associated floodplains (Whipple and Viers 2019). While there has been considerable progress in the science underpinning provision of flows for the environment (hereafter 'environmental flows') there remain substantive gaps in our understanding (Poff and Zimmerman 2010; Davies et al. 2014; Arthington et al. 2018). These include an understanding of the processes which link flow variability to fluxes of energy to enhance condition, fitness and recruitment of higher consumers such as fish and birds (Rolls et al. 2017).

Australia, the world's driest inhabited continent, has experienced profound water stress which has had substantive environmental, social and economic impacts. In south-eastern Australia the Murray–Darling Basin (MDB), Australia's largest food growing region, has experienced acute ecological impacts of water resource development (Kingsford 2000; Jenkins et al. 2005; Murray-Darling Basin Authority 2011a). In the MDB, the high level of water use has proven to be unsustainable, both in meeting the needs of irrigators for agriculture and for the ecological health of rivers and wetlands (Kingsford 2000; Arthington and Pusey 2003; Murray-Darling Basin Authority 2011b; Docker and Robinson 2014). Increasing the share of environmental water and improving environmental water management are central elements of current water reform in the MDB (Murray-Darling Basin Authority 2011b; Docker and Robinson 2014). A monitored program of delivering water dedicated to the environment to instream channels and wetlands has now been in place for seven years, providing the basis for internationally significant insights into ecological responses to environmental flows (Horne et al. 2018).

Environmental flows are used as a management tool to restore parts of the hydrological regime altered by human water use to rehabilitate the environment (Arthington 2012). These flows aim to restore overall ecosystem health, but frequently express targets in terms of population level outcomes in higher-order consumers such as iconic fish and bird species (Gawne et al. 2014). Research and monitoring to date has focused on understanding these 'flow-ecology' relationships, without investigating the mechanisms underlying them (Rolls et al. 2017). The production and transfer of trophic energy within a system is a process fundamental to the success of higher-order consumers (Lowe et al. 2006). A better understanding of the interaction between hydrological flow regimes, trophic dynamics and higher-order consumers would allow a more thorough process-based evaluation of environmental flows (Robson et al. 2017).

A key element of the transfer of energy through food webs is the quality of different food resources. Food quality affects the growth, development, and mortality of consumers (Rossi et al. 2006; Malzahn et al. 2007; Masclaux et al. 2012; Paulsen et al. 2014). For vertebrate consumers, fatty acids are especially important, as they are required for neural and visual development (Kainz et al. 2004; Masclaux et al. 2012). The fatty acids 18:3n-3 α -linolenic acid (ALA), and 18:2n-6 linoleic acid (LA), cannot be directly produced by heterotrophic organisms and must come from food (Arts et al. 2009). The

polyunsaturated fatty acids (PUFA) 20:5n-3 eicosapentaenoic acid (EPA), 22:6n-3 docosa-hexaenoic acid (DHA), and 20:4n-6 arachidonic acid (ARA) are required by all organisms and play a role in health and cell function (Dalsgaard et al. 2003). Fatty acids are chemically varied, are often incorporated unaltered into higher-order consumers, and different organisms can have distinct profiles (Arts et al. 2009). Fatty acids may therefore act as both dietary tracers in the food web and indicators of overall food quality (Iverson et al. 2004; Ravinet et al. 2010; Gonçalves et al. 2012; Magnone et al. 2015; Guo et al. 2017; Kohlbach et al. 2017).

Feeding trials in which invertebrates and fish were provided with various food sources of differing fatty acid composition generally indicate that quality ranges from bacteria (lowest) through cyanobacteria (moderate) to green algae (most nutritious) (Peltomaa et al. 2017). However there have been, to date, no studies of how environmental flows may influence resource availability and thus consumer growth and condition. We sought to understand how different food sources are incorporated into fish tissue in temporary wetland systems. We added different sources of fatty acids to artificial wetland mesocosms reflecting the inputs likely to occur due to environmental flows. These included; redgum (*Eucalyptus camaldulensis*) leaf leachate, cyanobacteria, green algae and biofilm matrix (comprising algae, bacteria and fungi). These were used to represent basal resources which are known to alter during provision of environmental flows (Wolfenden et al. 2018). Red gum is the dominant floodplain tree in the MDB, and leachate additions reflected scenarios where environmental water inundated leaf litter on in-stream benches or on the floodplain. Red gum leachate was intended to also increase planktonic bacteria through the provision of dissolved organic carbon (DOC). Cyanobacteria (e.g. Williamson et al. 2018), phytoplankton, green algae and biofilm availability can be altered both directly, and indirectly via nutrient inputs, during environmental flows (e.g. Sheldon and Walker 1997; Ryder et al. 2006).

This study provides a necessary first step toward the development of mechanistic research that investigates the effects of variation in FA within the food and prey base on top predators. Invertebrates and fish were placed in mesocosms treated with the different basal resource types. We hypothesised that 1. invertebrates and fish that were fed different food sources would attain differing FA profiles and 2. differences in the FAs available from those different food sources would result in differences in somatic growth of invertebrates and fish, ultimately resulting in differences in fish sizes and invertebrate community composition.

Methods

Mesocosm design

Mesocosm experiments were established at the University of New England (UNE) campus at Armidale, NSW. Soil was collected from a dry wetland (to a depth of approximately 10 mm) on the Gingham watercourse, New South Wales, Australia (29°16'S 149°21'E) in October 2017. Vegetation on the site is classified as water couch marsh grassland (Bowen and Simpson 2010; Southwell et al. 2015). Soil was transported to the UNE campus then passed through a 10-mm mechanical sieve for homogenization and to remove roots and vegetation. Fifteen 500-L vats with a basal surface area of 0.78 m² were placed in an open-ended structure covered with white polyethylene which allowed enough ambient light for algae and vegetation growth. Vats were filled to a depth of 300 mm with potable water, allowed to stand for three days to dechlorinate and maintained at that depth for the

remainder of the experiment. Ten kilograms of wetland soil was added at the end of the three days resulting in a soil depth of approximately 10 mm. The vats were gently aerated with diffusers. The vats were allowed to stand for 28 days prior to the introduction of fish in order to produce enough invertebrate biomass from sediments to sustain juvenile fish. Individual shield shrimp (*Triops australiensis*) were removed from the vats when observed, since a pilot study and (Boulton and Lloyd 1992) had shown that either their activity within the soils or their feeding behaviour reduced the populations of other invertebrates of suitable prey size for small fish.

Young Western carp gudgeons (*Hypseleotris* spp.) were collected in February 2018 from Tarban Creek (28°58'21"S; 151°54'1"E), using bait traps (360 × 240 × 240 mm, 5 mm mesh) set overnight. Fish were transported to the mesocosms, where eight individuals were randomly selected and added to each vat.

One of three potential food sources was added daily to each of three replicate vats; 1/ cyanobacteria as commercially available dried *Aphanizomenon flos-aquae* powder, 2/ green algae as commercially available dried *Chlorella* powder and 3/dried biofilm powder. In addition, three vats were dosed with dissolved organic carbon (DOC) every four to five days and three vats with no added food sources acted as a control treatment. Dissolved organic carbon (DOC) was sourced from river redgum (*Eucalyptus camaldulensis*) leaf leachate following the methods of O'Connell et al. (2000). The leachate was produced by submerging 800 g of dry leaves in 45 L of chlorinated potable water for 72 hrs at 3 °C, to reduce the consumption by bacteria until added to the vats. The biofilm was collected from wooden redgum tiles that were incubated within the photic zone of a 1 ML dam for six weeks, dried at 40 °C to constant weight and ground to a fine powder. Seventy micrograms of powder was added to the cyanobacteria treatments, 30 µg of powder to the green algae treatments and 70 µg of powder to the biofilm treatments. The amount of cyanobacteria and green algal powder added to each vat was calculated to increase the chlorophyll *a* (Chla) concentration by 4 µg/L, effectively doubling the average concentration identified in a previous mesocosm experiment (Growth, unpublished data).

Sampling of the mesocosms

Prior to the introduction of the gudgeons planktonic and benthic invertebrates were sampled to estimate abundance and assemblage structure. Zooplankton were sampled by haphazardly taking five separate 200 ml aliquots of mesocosm water from each tank, combined, preserved in ethanol and stained with rose Bengal. Benthic invertebrates were sampled following King (2004) by taking one core (50 mm diameter x 120 mm long with 250 mL volume) for each tank, put through a 250 µm sieve, preserved in ethanol and stained with rose Bengal. Fourteen days after the introduction of fish planktonic and benthic invertebrates were again sampled from each vat using the same methods. Additional plankton and benthos were collected using a 400 µm net to provide enough material for fatty acid profiling from each vat prior to them being drained to remove the remaining gudgeons. Following the draining of the vats organic components of the sediments were collected by elutriation with water and passed through a series of sieves to obtain coarse particulate organic matter (CPOM; 400 µm to 250 µm), fine particulate organic matter (FPOM; 250 µm to 125 µm) and very fine particulate organic matter (VFPOM; 125 µm to 63 µm).

Gudgeons were anaesthetised, measured for standard length, weighed and their entire alimentary canal removed and preserved in ethanol for gut content analysis. Invertebrates

were identified to Genus for clam shrimp, shield shrimp and snails (Spinicaudata, Notostraca and Gastropoda, respectively), Family for true flies (Diptera) and water-fleas (Cladocera), Order for copepods (Copepoda), Class for seed shrimp (Ostracoda) and Phylum for roundworms (Nematoda) and rotifers (Rotifera).

Gut content analysis

The contents of the alimentary canal of each fish captured at the end of the experiment were spread out on a small petri dish and examined under a binocular microscope. All taxa were counted and identified to the taxonomic level outlined in the previous section, with the exception of Macrothricidae and Daphnidae, which were pooled as most of them could not be distinguished due to partial digestion of their exoskeletons.

Fatty acid analyses

Profiling of FA followed the methods used by Conlan et al. (2017). Briefly, lipid was extracted from dry samples soaked dichloromethane: methanol (CH₂Cl₂:CH₃OH) and quantified gravimetrically on a 4-digit balance. FA were extracted following lipid class analysis determined using an Iatroscan MK 6s thin layer chromatography-flame ionisation detector. Following extraction, FA were esterified into methyl esters using the acid catalysed methylation method (Christie 2003). Gas chromatography was then used to identify the FA methyl esters relative to known external standards. FA profiling was undertaken on benthic and pelagic invertebrates, gudgeons taken from the start and finish of the experiment, the three substratum size fractions from each of the three replicate mesocosms and the algal and biofilm powders.

To determine the primary food source in gudgeons at the end of the experiment we used the following individual FA as biomarkers, myristic acid (14:0) for cyanobacteria (Carpenter et al. 1997; Kelly and Scheibling 2012), pentadecylic acid (15:0) and margaric acid (17:0) for bacteria (Dalsgaard et al. 2003; Alfaro et al. 2006), oleic acid (18:1n-9) for fungi (Vestal and White 1989; Willers et al. 2015), alpha-linolenic acid (18:3n-3) for green algae (D'Souza and Loneragan 1999; Kelly and Scheibling 2012) and eicosapentaenoic acid (20:5n-3) for diatoms (Volkman et al. 1989). The nutritional value of various food sources was defined as the sum of n-3 and n-6 PUFA.

Fish dietary preference

We used the Manly-Chesson index α (Manly 1974; Chesson 1978, 1983) to assess prey selection. The equation for the Manly-Chesson index is as follows:

$$\alpha_i = \frac{r_i/p_i}{\sum_{i=1}^m r_i/p_i}$$

where r_i is the relative abundance of prey taxon ' i ' found in the larval diet, p_i is the relative abundance of the same prey item found in the environment and m is the number of food items. A value less than $1/m$ indicates a prey group that was consumed disproportionately less than its relative abundance in the environment. Values near $1/m$ indicate that a prey taxon was consumed in direct proportion to its abundance, and values greater than $1/m$ indicate a prey group was consumed disproportionately more than its relative abundance in the resource base, with values near 1.0 indicating a strong selection of a

prey item or 'preference'. Separate calculations were performed for the planktonic and benthic invertebrates in each treatment.

Statistical analyses

All statistical analyses were conducted using PERMANOVA+ for PRIMER software (Anderson et al. 2008). Permutational analysis of variance (PERMANOVA) (Anderson 2001) was used to identify significant sources of variation among factors (Supplementary material Table S1) using 9999 randomisations. Bray–Curtis dissimilarity was used to define distances among samples for multivariate data (e.g. FA profiles or invertebrate assemblages) and Euclidean distance for the univariate data. Invertebrate abundance data was converted to ranks within each habitat type and Kendall rank correlation coefficients were used to form the similarity matrix for PERMANOVA analysis. The homogeneity of variance, that is equal dispersion, among groups of the main factors was tested using PERMDISP (Anderson 2001). Patterns of differences in invertebrate assemblages and fatty acid profiles identified by PERMANOVA were presented graphically using bootstrap averaging ordination with 100 randomized starts (Clarke et al. 2014). The variables responsible for the separation of significantly different groups in multivariate data were tested using similarity percentages (SIMPER) in the PRIMER software (Clarke 1993). The consistency ratio (the ratio of the average to standard deviation of the dissimilarities between groups) was calculated for each species that had a greater than 3% contribution to the average dissimilarity.

Results

Fatty acid profiles

A total of 47 individual fatty acids were identified from the food sources, substratum fractions and animal groups (Supplementary material Table S2). Palmitic acid (16:0) was present in greater than 20% of all samples and oleic acid (18:1n-9) greater than 10% in all the substratum fractions, green algae and biofilm (Supplementary material Table S2). At commencement of our mesocosm experiment gudgeon tissue contained palmitic acid and oleic acid in similar proportions to the substratum fractions, however the proportion of oleic acid among total lipids increased in gudgeon tissue to more than 15% by the end of the experiment. Palmitic acid accounted for more than 30% of total lipids among all benthic invertebrate samples, while stearic acid (18:0) occurred in proportions above 15%, with no other single fatty acid accounting for more than 10% of total lipids. Planktonic taxa also had palmitic acid and oleic acid in proportions exceeding 10% of total lipids as well as two additional fatty acids alpha linolenic acid (ALA, 18:3n-3) and palmitoleic acid (16:1n-7). The cyanobacteria food source differed from the other two food sources by having the greatest percentage of palmitic acid, myristic acid (14:0) and tetracosapentaenoic acid 24:5n-3. Biofilm had the greatest percentage of stearic acid and oleic acid (18:1n-9). In contrast, green algae had the highest concentration of alpha-linolenic acid (18:3n-3).

The fatty acid profiles of the different groups (substratum fractions and animal groups) and between treatments were significantly different with no interaction between those factors (Table 1). Pairwise tests demonstrated fatty acid profiles of each group differed significantly from one another (Figure 1). In addition, the variation within groups differed significantly between the different groups, with fish demonstrating little variation

Table 1. Pseudo-F values and probability levels for fatty acid profiles and food source biomarkers for differences between groups (substratum fractions and biotic groups) and mesocosm treatments.

Total profile and food source biomarkers	Group (G)		Treatment (T)		G x T interaction
	PERMANOVA	PERMDISP	PERMANOVA	PERMDISP	PERMANOVA
Total profile	25.2 ($p = 0.0001$)	9.7 ($p = 0.0001$)	2.3 ($p = 0.026$)	ns	ns
Cyanobacteria	28.2 ($p = 0.0001$)	5.1 ($p = 0.0261$)	ns	na	ns
Bacteria	11.1 ($p = 0.0001$)	ns	4.2 ($p = 0.0001$)	ns	ns
Green algae	22.7 ($p = 0.0001$)	10.8 ($p = 0.0001$)	ns	na	ns
Fungi	23.1 ($p = 0.0001$)	ns	3.6 ($p = 0.0106$)	ns	ns
Diatom	42.4 ($p = 0.0001$)	25.1 ($p = 0.0001$)	ns	na	ns

compared with benthic invertebrates and zooplankton (Figure 1, Table 1). The fatty acid profiles from the different treatments of the different groups (substratum fractions and animal groups) were all significantly different from each other with the exception that the control treatments were not different from the DOC and green algal treatments and the green algal treatment was not different to the biofilm and BGA treatments.

The mean percentage of each of the basal source biomarkers differed between groups and the bacterial and fungal biomarkers differed between treatments, although the low pseudo-F values indicated that only a small fraction of the variance was explained by treatment (Table 1). The cyanobacterial fatty acid biomarker (myristic acid) was highest in the benthic invertebrate group regardless of treatment (Supplementary material Figure S1). In contrast, the mean percentage of the fungal biomarker (oleic acid) was lowest in the benthic invertebrates. In general, the mean percentage of green algal and diatom biomarkers (alpha-linolenic and eicosapentaenoic acids, respectively) was highest in the planktonic invertebrates, followed by the fish.

Fatty acid profiles of gudgeons at the start of the experiment were significantly different to fatty acid profiles of gudgeons from all treatments at the end of the mesocosm experiment (Figure 2, Table 2). However, there was no significant difference in the fatty acid profiles of gudgeons in treatments at completion of the experiment. SIMPER analysis suggested that three fatty acids contributed to the differences between gudgeons at the beginning and the end of the experiment. These included oleic acid (18:1n-9) and arachidonic acid (ARA, 20:4n-6) which increased from 10.8% to 15.6% and 4.7% and 7.6% respectively and palmitoleic acid (16:1n-7) which decreased from 8.6% to 5.8%.

The fatty acid biomarkers for bacteria and fungi differed between the fish groups (Table 2). Pairwise tests indicated that, at the start of the experiment gudgeons had a significantly greater mean percentage of the bacterial markers than at the end (Figure 2). In contrast, the fish at the start of the experiment had significantly lower mean percentages of the fungal biomarker than at the end.

Nutritional values

Nutritional value (sum of n-3 and n-6 PUFA) differed significantly between groups (substratum fractions and animal groups) ($F_{5,54} = 51.5$, $p = 0.0001$) but not between treatments and there was no significant interaction between factors. In addition, there was significant heterogeneity between groups ($F_{1,5} = 3.6$, $p = 0.0115$) with fish having the lowest variation among samples and the zooplankton the greatest. Pairwise tests indicated that zooplankton and fish had the highest nutritional values of the six groups (Figure 3).

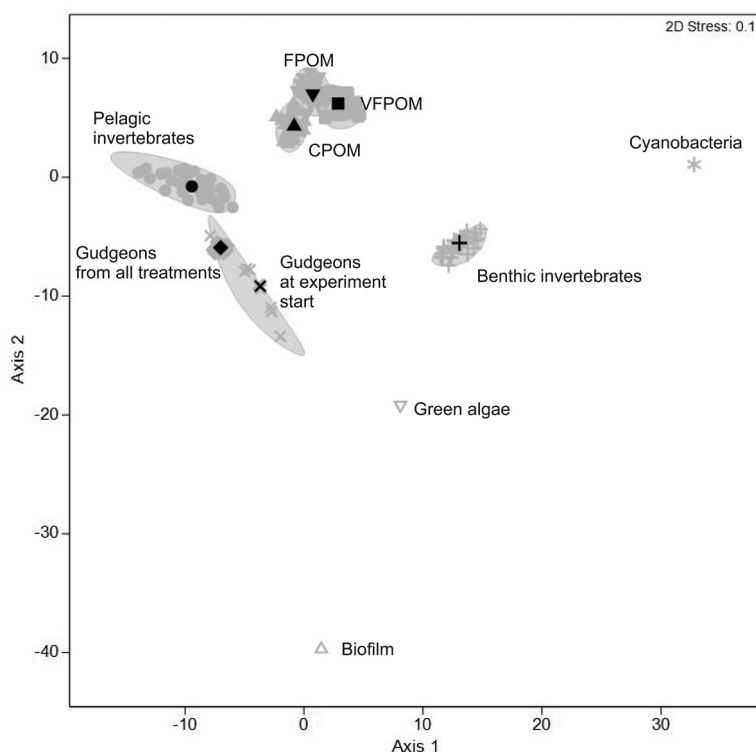


Figure 1. Bootstrap average ordination of fatty acid profiles of the different groups. Solid symbols represent average locations for particular groups. Ellipses represent regions in which 95% of these bootstrap averages fall for each group.

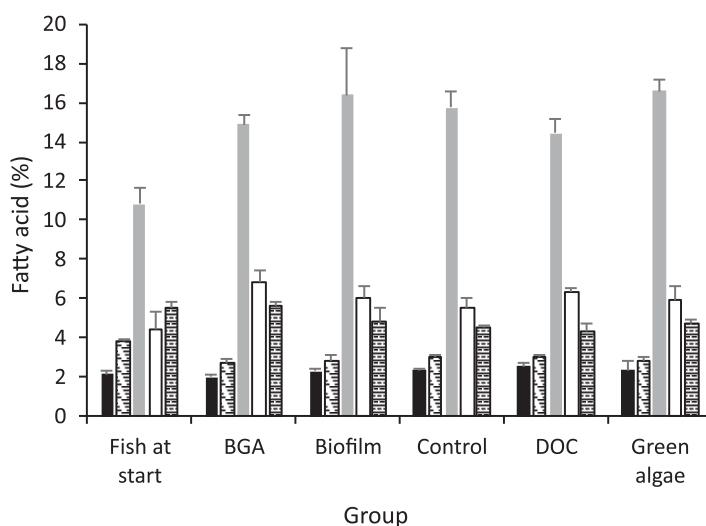


Figure 2. Mean percentage (± 1 S.E.) of fatty acid biomarkers for different possible basal food sources among six gudgeon treatments. Black columns indicate the cyanobacterial biomarker, striped indicate bacterial markers, grey indicate the fungal marker, white indicate the green algal biomarker and spotted the diatom biomarker.

There was a significant difference in the nutritional value of fish among treatments (including fish at the start of the experiment) (pseudo $F_{5,12} = 3.5$, $p = 0.0249$), with no significant heterogeneity between treatments. Fish at the start of the experiment

Table 2. Pseudo-F values and probability levels for fatty acid profiles and basal source biomarkers for differences between fish groups.

Total profiles and food source biomarkers	PERMANOVA	PERMDISP
Total profile	2.3 ($p = 0.0095$)	ns
Cyanobacteria	ns	na
Bacteria	4.8 ($p = 0.0195$)	ns
Green algae	ns	na
Fungi	3.3 ($p = 0.040$)	ns
Diatom	ns	na

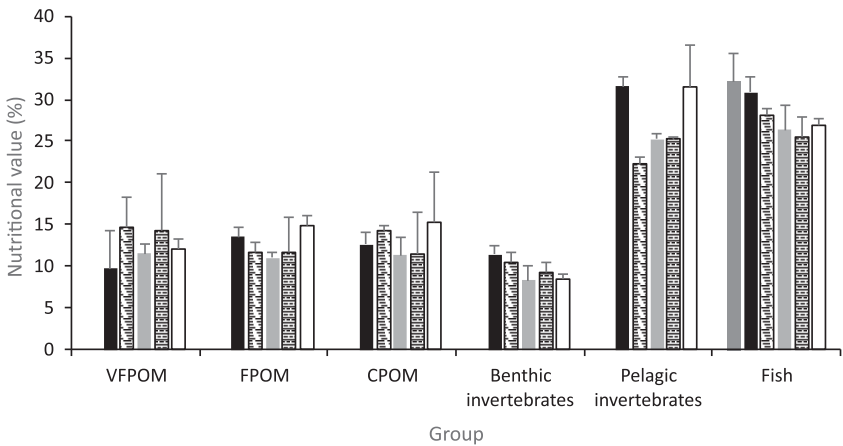


Figure 3. Mean nutrition value (± 1 S.E.) of substratum fractions and animal groups. Black columns the BGA treatment, striped columns biofilm, light grey control mesocosms, dotted columns DOC and white columns green algae treatments. Dark grey column represents fish at the start of the experiment.

had a significantly higher nutritional value than fish in the remaining treatments (Figure 3).

Invertebrate assemblage structure

A total of 4264 invertebrates from seven taxa were collected from benthic and planktonic habitats during the course of the experiment. Approximately half of the animals were Daphniidae (49%), and were followed in abundance by Macrothricidae (29%), Ostracoda (12%) and Chironomidae (9%). Chydoridae, Harpacticoida and Nematoda comprised less than one percent of the total abundance.

Time and habitat were the main sources of variation in the invertebrate assemblage structure, explaining 32% and 35%, respectively (Table 3). The only other significant source of variation was the habitat by treatment interaction. Post-hoc pairwise tests indicated that the assemblage structure in the DOC and biofilm treatments differed significantly ($p < 0.05$) from the green algae treatment in the benthic habitat but all the other comparisons between treatments in the benthic and planktonic habitats were not significantly different.

Both benthic and planktonic invertebrate communities showed clear shifts in composition from prior to introduction of gudgeons to the mesocosms to those at the end (Figure 4). Taxa contributing most to dissimilarity between benthic and planktonic assemblages included Chironomidae, Macrothricidae, Ostracoda and Daphniidae (Table 4). The ranked abundance of all taxa with the exception of Daphniidae was greater in the

Table 3. Results of PERMANOVA for invertebrate assemblage structure.

Source	df	Pseudo-F	P(perm)	% variance	PERMDISP
Time (Ti)	1	26.8	0.0001	32	ns
Habitat (H)	1	29.6	0.0001	35	ns
Treatment (Tr)	4	1.2	0.3303	1	ns
Ti x H	1	0.0	0.9900	0	na
Ti x Tr	4	1.9	0.0909	6	na
H x Tr	4	2.4	0.0370	9	na
Ti x H x Tr	4	0.3	0.8488	0	na

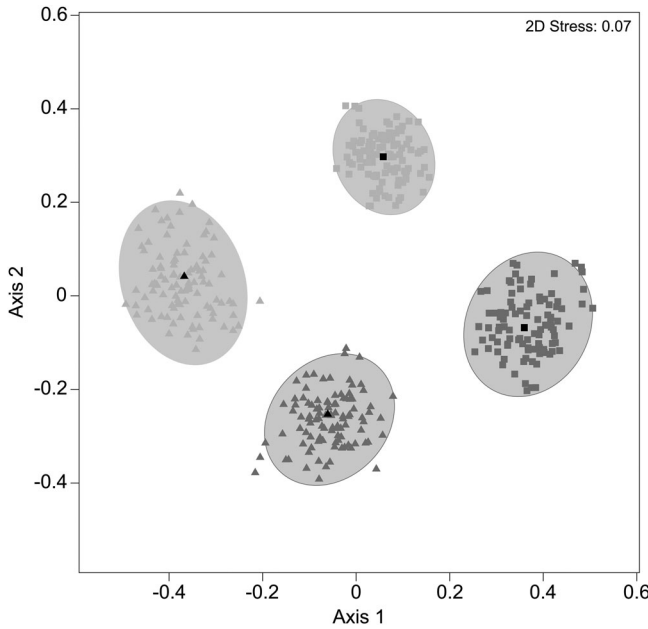


Figure 4. Bootstrapped average ordination of invertebrate assemblages in benthic and planktonic habitats before of the introduction of gudgeons into the mesocosms and at the finish of the experiment. Triangles represent benthos and squares planktonic samples. Light grey represents samples prior to the introduction of fish and darker grey invertebrates at the finish of the experiment. Ellipses represent regions in which 95% of these bootstrap averages fall for each group.

Table 4. Mean ranked abundance and percentage contribution of taxa contributing to the differences between (a) habitat types and (b) time as indicated by SIMPER analysis.

(a) Taxon	Benthos	Plankton	Contribution (%)
Chironomidae	14.9	1.0	22.0
Macrothricidae	15.0	13.1	20.8
Ostracoda	15.0	12.9	19.8
Daphnidae	13.9	16.1	19.5
(b) Taxon	Start	Finish	Contribution (%)
Macrothricidae	19.7	8.5	25.1
Daphnidae	9.8	20.3	22.6
Ostracoda	15.0	12.9	19.7
Chironomidae	7.2	8.6	15.0

benthos. The taxa associated with the differences in assemblage structure through time included the same four taxa associated with the differences between habitats (Table 4). The ranked abundance of Chironomidae and Daphniidae increased though time compared with the other two taxa.

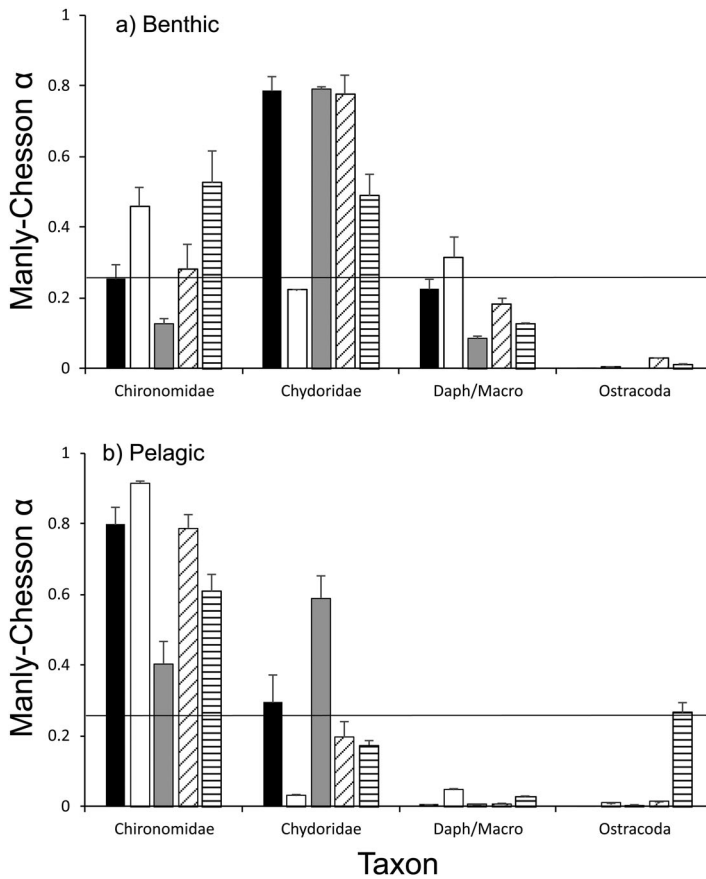


Figure 5. Mean (± 1 S.E.) Manly-Chesson α for different habitats, taxa and treatments. Black columns indicate the cyanobacteria treatment, white columns control, grey columns dissolved organic carbon, diagonal striped columns green algae and horizontal lines the biofilm treatment. The horizontal line is the neutral value for selectiveness.

Western carp gudgeons and gut content analysis

Eighty six fish (72%) were recovered from the mesocosms. There was no significant difference in either survival or weight of fish between treatments (pseudo $F_{4,10} = 0.8$ and 1.2 , respectively, $p > 0.05$).

A total of 1212 invertebrates from five taxa were identified from the gut contents of the fish. The majority of the invertebrates were Chironomidae (44%) followed by Daphniidae (43%), Chydoridae (11%) and Ostracoda (2%). There were no significant differences in the proportion of dietary items between treatments (pseudo $F_{4,10} = 1.5$, $p = 0.21$).

The mean Manly-Chesson index α differed significantly between taxa (pseudo $F_{3,80} = 31.9$, $p = 0.0001$) but interacted significantly with habitat and treatment (pseudo $F_{3,80} = 11.6$ and $F_{12,80} = 3.0$, respectively, $p = 0.0001$). Pairwise tests indicated that the mean α for Chironomidae, Chydoridae and Daphniidae/Macrothricidae differed significantly between habitats but not for Ostracoda. The index was greater for Chironomidae in the planktonic habitat compared with the benthic habitat (Figure 5). In contrast, the index was greater in the benthic habitat for Chydoridae and the pooled Daphniidae and Macrothricidae. The significant taxon by treatment interaction was most likely the result of a significant difference in mean α between the control and DOC treatments for

Chironomidae and Chydoridae but not the other two taxa. Mean α was greater in the control treatment compared with the DOC treatment for Chironomidae in both habitats (Figure 5). In contrast, the index was greater in the DOC treatment compared with the control in both habitats for Chydoridae.

In general, the mean α was greater than the neutral value for selectiveness for Chironomidae in the planktonic habitat and Chydoridae in the benthic habitat (Figure 5). This indicates that the gudgeons were selecting different taxa in different habitats. In contrast, the mean α was less than the neutral value for selectiveness for both Daphniidae/Macrothricidae and Ostracoda in both habitats, suggesting that gudgeons are avoiding those prey.

Discussion

There was a clear shift in the invertebrate assemblage structure following the introduction of the gudgeons. There was also a clear difference in assemblage structure and nutritional value between habitat types. In addition, the gudgeons appeared to target different taxa in the different habitat types, Chironomidae from the plankton and Chydoridae from the benthos and avoided Ostracoda and Daphniidae/Macrothricidae in both habitat types. Western carp gudgeons are known to eat Daphniidae, Chironomidae and to a lesser extent ostracods and chydorids (Meredith et al. 2003; Balcombe and Humphries 2006; Balcombe and Closs 2016). Through their dietary preferences gudgeons can alter the assemblage structure of zooplankton (Ning et al. 2010) but it is unclear if this is the case for benthos. Young gudgeons are known to inhabit benthic and pelagic habitats (Stoffels and Humphries 2003) so it is possible that the change in benthic assemblage structure was the result of gudgeon feeding preferences. It is unclear why the gudgeons were selectively feeding in our mesocosms. It may be that the different taxa have different nutritional values but overall the planktonic invertebrates were more nutritious in terms of total PUFA. Experiments which involve the addition of different food sources to wetland mesocosms are rare. We are aware of only one other study, Williams et al. (2007) who added extra leaf material and algal cultures to pond mesocosms. However, similar to our results they found few differences in the invertebrates colonizing the different treatments. This may suggest that food sources generated within the mesocosms themselves may have outweighed any treatment effects.

The addition of extra food sources did not generate differing FA profiles between treatments in the substratum fractions, invertebrates or fish. This is despite the FA profiles of the food sources added differing and being indicative of their different biomarkers; 14:0 for cyanobacteria, 18:3n-3 for green algae and 18:1n-9 for fungi in the biofilm. This suggests that food sources generated within the mesocosms themselves may have outweighed any treatment effects. The significant increase in the fungal FA biomarker in all treatments suggests that the main food source in the mesocosms is derived from fungi. The use of FA as biomarkers is commonplace in the food web literature (e.g. Johns et al. 1979; Goedkoop et al. 2000; Falk-Petersen et al. 2002). However, there are some specific FA that have been attributed to different biotic groups by different authors. For example, the FA 16:0 has been used as a biomarker for green algae, cyanobacteria and fungi (Vestal and White 1989; Kelly and Scheibling 2012). Biomarkers for specific species of algae are easily overlapped, such as the 18:3n6 has been simultaneously used as a marker for green algae (Meziane and Tsuchiya 2000; Xu et al. 2014) and cyanobacteria (Hayakawa et al. 2002; de Kluijver et al. 2012). In addition, the one biomarker for a particular group may not be present for environmental reasons. The FA 20:5 $\times$ 3 although an

often consistent biomarker for diatoms is often not found in large quantities relative to other FA (Napolitano 1999) and may be found in lower light or nutrient concentrations (Ahlgren et al. 1992). Given biomarkers for individual species of plankton, aquatic and terrestrial materials are not always specific enough to identify a single source and the fact that the internal biosynthetic capabilities of most organisms have not been elucidated, some authors caution their use in food web studies (Wakeham 1995; Dalsgaard et al. 2003).

An explanation for the lack of difference in Western carp gudgeon size or survival and FA profiles between treatments may be that the mesocosm experiment was run for insufficient time for any changes to manifest themselves. However, three fatty acids contributed to the differences between gudgeons at the beginning and the end of the experiment. These included increases in oleic acid and ARA and a decrease in palmitoleic acid suggesting that there was sufficient time for changes to occur. In addition, a previous mesocosm experiment involving larval Murray cod demonstrated two weeks was sufficient time for changes in FA profiles (Grown, unpublished data).

Food web and diet studies that attempt to identify the mechanisms for basal food source uptake and conversion generally only occur from the source itself to primary consumers (Taipale et al. 2012; Freese and Martin-Creuzburg 2013). In these studies the biota are mainly cladoceran species fed specific food sources, i.e. animals cannot choose between food types. Linking FA profiles from basal food sources through primary consumers to secondary consumer are rare and have only occurred in marine systems (Oberg and Fuiman 2015). Previous field investigations of fish FA composition in freshwater systems have mainly focused on temporal and spatial changes e.g. Dayhuff and Wells (2005), and a direct, quantitative comparison of fish FA profile to their food sources has yet to be made (Guo et al. 2017). Our mesocosm experiment attempted to identify the fate of different primary production sources through primary and then secondary consumers by providing alternate sources of food in addition to that generated from flooding dry wetland sediment. Using flooded wetland mesocosms potentially would have provided more realistic knowledge of the food web mechanisms of wetland inundation rather than feeding zooplankton fed specific primary food sources to fish. However, future experiments attempting to identify the mechanisms of the transfer of basal food sources in wetlands to secondary consumers may wish to directly feed fish primary consumers raised on specific basal food sources.

Acknowledgements

Michael Faint from University of New England is thanked for providing space in the agronomy polytunnel for the mesocosms. David Francis from Deakin University is thanked for organising the fatty acid profile analyses. Simon Mitrovic from University of Technology Sydney is thanked for organising the counting of bacteria. Sam Lewis is thanked for assisting with the collection of wetland soils. Lindsey Frost made useful comments on earlier drafts of the manuscript.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This research forms part of and was funded by the Environmental Water Knowledge and Research Project which is funded by the Australian Government's Department of the Environment and Energy and coordinated by the Murray–Darling Freshwater Research Centre. All work was conducted under the UNE

Animal Ethics Committee Authority No.: AEC17-091 and NSW Department of Primary Industries Scientific Collection permit P17/0056-1.0 & OUT17/34715. Data from the mesocosm experiment is freely available at [10.4121/uuid:2f1210af-2898-4330-93ad-96d934e7fcf2](https://doi.org/10.4121/uuid:2f1210af-2898-4330-93ad-96d934e7fcf2).

Notes on contributors

Ivor Gowns is a Research Fellow at the University of New England, where he has conducted research on food webs and environmental flows.

Darren Ryder is a Professor at the University of New England, where he leads the Aquatic Ecology and River Restoration Research Group.

Paul McInerney is a Research Scientist at the Commonwealth Scientific and Industrial Research Organisation, where his primary interest is in energy flow within aquatic ecosystems.

Nick Bond is a Professor at LaTrobe University, where he studies the effects of flow variability on riverine ecosystems.

Galen Holt is an Associate Research Fellow at Deakin University, where his primary focus on the effects of variation on populations, communities, and ecosystems.

Rebecca Lester is an Associate Professor at Deakin University, where she studies the function and structure of ecosystems and in the development of management strategies that incorporate good ecological outcomes with human activities.

Ross Thompson is Director and Professor in the Centre for Applied Water Science at the University of Canberra, where his fundamental research is in food web ecology.

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