Appendix 1: Research Activity Reports

Appendix 1.1 Review and conceptualisation

The review and conceptualisation phase produced three manuscripts. Abstracts and links for each are included below.

Rolls, R. J, Baldwin, D. S, Ryder, D. S, Bond, N. R, Gawne, Lester, R. E, McInerney, P. J, Robson, B J, Thompson, R. M. Hydrological drivers of trophic dynamics in river and floodplain ecosystems as a basis for process-based restoration using environmental flows. In Prep.

Abstract

Environmental flows are a management tool used to restore aspects of the natural hydrological regime of river and floodplain ecosystems in order to support ecological processes that sustain populations and ecosystems. Existing conceptualisations of river and floodplain ecosystem function (e.g. River Continuum Concept, Flood Pulse Concept, Riverine Productivity Model) emphasise different mechanisms that affect sources and rates of primary production. Identifying the role of hydrology in structuring the trophic dynamics underpinning food webs that support higher order consumers in river and floodplain ecosystems has not directly been considered in these conceptual models. We ask four questions essential to understanding the effects of hydrology on trophic processes in river and floodplain systems and use a synthesis of empirical evidence to determine how well each can currently be answered. These questions relate to the effects of hydrology on (1) the sources and production of organic matter, (2) transport and accessibility of energy throughout river and floodplain systems, (3) nutritional value of energy resources for consumers, and (4) the transfer of energy through food chains to higher consumers. To further develop environmental flows as a tool for process-based restoration there is a need to distinguish and integrate multiple interactions between hydrology and trophic dynamics. Framing environmental flows around principles of process-based restoration also highlights research needs in the areas of context-dependency and spatial and temporal scales of the effects of hydrology on trophic dynamics in river and floodplain systems.

Rolls, R.J., Baldwin, D.S., Bond, N.R., Lester, R.E., Robson, B.J., Ryder, D.S., Thompson, R.M., and Watson, G.A. (2017) A framework for evaluating food-web responses to hydrological manipulations in riverine systems. Journal of Environmental Management 203, 136-150.

Link to full publication: https://www.sciencedirect.com/science/article/pii/S030147971730717X

Abstract

Environmental flows are used to restore elements of the hydrological regime altered by human use of water. One of the primary justifications and purposes for environmental flows is the maintenance of target species populations but, paradoxically, there has been little emphasis on incorporating the food-web and trophic dynamics that determine population-level responses into the monitoring and evaluation of environmental flow programs. We develop a generic framework for incorporating trophic dynamics into monitoring programs to identify the food-web linkages between hydrological regimes and population-level objectives of environmental flows. These linkages form the basis for objective setting, ecological targets and indicator selection that are necessary for planning monitoring programs with a rigorous scientific basis. Because there are multiple facets of trophic dynamics that influence energy production and transfer through food webs, the specific objectives of environmental flows need to be defined during the development of monitoring programs. A multitude of analytical methods exist that each quantify distinct aspects of food webs (e.g. energy production, prey selection, energy assimilation), but no single method can provide a basis for holistic understanding of food webs. Our paper critiques a range of analytical methods for quantifying attributes of food webs to inform the setting, monitoring and evaluation of trophic

outcomes of environmental flows and advance the conceptual understanding of trophic dynamics in river-floodplain systems.

Robson, B.J., Lester, R.E., Baldwin, D.S., Bond, N.R., Drouart, R., Rolls, R.J., Ryder, D.S., and Thompson, R.M. (2017) Modelling food-web mediated effects of hydrological variability and environmental flows. Water Research 124, 108-128.

Link to full publication: https://www.sciencedirect.com/science/article/pii/S0043135417306048

Abstract

Environmental flows are designed to enhance aquatic ecosystems through a variety of mechanisms; however, to date most attention has been paid to the effects on habitat quality and life-history triggers, especially for fish and vegetation. The effects of environmental flows on food webs have so far received little attention, despite food-web thinking being fundamental to understanding of river ecosystems. Understanding environmental flows in a food-web context can help scientists and policy-makers better understand and manage outcomes of flow alteration and restoration. In this paper, we consider mechanisms by which flow variability can influence and alter food webs and place these within a conceptual and numerical modelling framework. We also review the strengths and weaknesses of various approaches to modelling the effects of hydrological management on food webs. Although classic bioenergetic models such as Ecopath with Ecosim capture many of the key features required, other approaches, such as biogeochemical ecosystem modelling, end-to-end modelling, population dynamic models, individual-based models, graph theory models, and stock assessment models are also relevant. In many cases, a combination of approaches will be useful. We identify current challenges and new directions in modelling food-web responses to hydrological variability and environmental flow management. These include better integration of food-web and hydraulic models, taking physiologically-based approaches to food quality effects, and better representation of variations in space and time that may create ecosystem control points.

Appendix 1: Research Activity Reports

Appendix 1.2 Fish field program

Included below is the full draft manuscript under review with the journal Limnology and Oceanology. It details the full work component completed under the fish field program activity within the food web theme.

Basal resource quality and energy flow in a lowland river food web

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Running Head: Energy flow in a lowland river food web

Keywords: Fatty acids, seston, basal energy, food web, lowland river, stable isotopes, wetlands, floodplain, anabranches, PUFA

Abstract

Understanding energy flow through ecosystems and among sub-habitats is critical to understanding patterns of biodiversity and ecosystem function. It can also be of considerable applied interest in situations where managing for connectivity among habitats is important to restoring degraded patterns of productivity. Here, we describe patterns of basal energy flow in different habitats in a lowland river in the Murray River catchment, Australia. The three habitats - river channels, anabranches and wetlands - are of particular interest as substantive management effort is currently being expended to connect these habitats using environmental flows, which seek to restore critical functional components of natural hydrological regimes, including such inter-habitat connections. A combination of stable isotopes and fatty acids were used to determine the source of energy and energy pathways through food webs across the three habitats. We found clear differences in the quality of basal resources between the river channel and floodplain habitats. Floodplains were characterized by food resources with higher concentrations of essential fatty acids. We show that inundation of floodplains and subsequent reconnection to the river is critical to: 1/ mobilise high quality food resources to the main channel; and 2/ to afford riverine consumers the opportunity to access to high quality resources by moving onto the floodplain. This research shows the importance of determining both the quantity and quality of organic matter fluxes into food webs, and the potential role of targeted environmental flows to re-establish critical energy pathways in riverine ecosystems.

Introduction

Food webs are fundamental to ecosystem function and describe the energy pathways between resources and consumers (Hladyz et al. 2011; Winemiller 1996; Woodward and Hildrew 2002). Within freshwater ecosystems research has focused on understanding the contribution and importance of terrestrially- and aquatically-derived carbon to food webs (Bunn and Boon 1993; Doucett et al. 2007; Nakano et al. 1999), with less attention oriented towards determining the quality of food resources (Guo et al. 2016a; Twining et al. 2017) and the dominant pathways for energy to reach higher trophic levels (e.g. Cashman et al. 2016; Taipale et al. 2011). Understanding which resources underpin food webs and factors influencing their availability to consumers is central to improving our capacity to gauge ecosystem health (Brett et al. 2017).

Food quality, in its coarsest form, may be assessed by ecological stoichiometry (e.g. C:N, Elser et al. 2000), however consumers can be limited by availability of complex organic compounds such as amino acids (Dwyer et al. 2018), sterols and fatty acids (Twining et al. 2016). Within freshwater ecosystems, algae and, in particular, diatoms are considered high-quality food resources for herbivorous taxa due to their high concentration of nutrients (nitrogen and phosphorous) and long–chain poly-unsaturated fatty acids (LC–PUFA) (Brett and Müller-Navarra 1997; Guo et al. 2015; Hill et al. 2011). Some omega–3 (ω3) and omega– 6 (ω6) PUFAs, such as eicosapentaenoic EPA (20:5ω3), docosahexaenoic DHA (22:6ω3) and arachidonic acid ARA (20:4ω6) are essential for physiological functions of consumers (e.g. somatic growth and reproduction, Sargent et al. 1999, Table 1). These compounds, often referred to as essential fatty acids, are not able to be synthesized by animals *de novo,* and must be either sourced from their diet (Parrish 2009) or synthesized from shorter-chain PUFA, such as alpha-linolenic (ALA; 18:3ω3) and linoleic acid (LIN; 18:2ω6), which

are plentiful in green algae and terrestrial plants (Guo et al. 2016a; Guo et al. 2016b). Gaining a better understanding of the spatial and temporal distribution and availability of these compounds within floodplain landscapes is important for assessing effects of changes in availability on higher consumers, such as fish and water birds.

Traditionally, ecologists have used stable isotope signatures to trace energy patterns in food webs (Giling et al. 2009), but this approach can have limitations where potential dietary sources have similar signatures (e.g. terrestrial litter, bacteria, seston) leading to difficulty in discriminating pathways (Baring et al. 2018). As such, diet-specific markers are increasingly used to help understand the relationship between resources and consumers and the flow of material through food webs (Taipale et al. 2013). The biomarkers selected are, preferably, unique to a particular food resource, easily determined, metabolically stable and not harmful to the organism (Dalsgaard et al. 2003), however meeting all of these conditions concurrently is rare (Taipale et al. 2011). Fatty acid composition has been shown to meet several of these criteria and has been widely used in both marine and freshwater food webs to trace energy pathways (Johansson et al. 2016; Kelly and Scheibling 2012) and to unravel trophic dynamics (Brett et al. 2009).

River–floodplain connectivity is known to strongly influence the productivity (Bayley 1991; Junk et al. 1989) and energy dynamics (Humphries et al. 2014) of aquatic ecosystems, but the specific role of floodplain wetlands and inter-connecting channels in supplying essential food elements to river consumers is less well understood. Temperate fish species are known to exploit inundated floodplain habitats and exhibit increased rates of somatic growth compared with growth in non-flood years (Gutreuter et al. 1999). In addition, fish productivity has been positively related to flood volume and also flooding extent (van de Wolfshaar et al. 2011). Terrestrially-derived carbon and nutrients released from sediments following floodplain inundation are known to increase aquatic invertebrate consumer biomass (McInerney et al. 2017), but the pathways by which consumers receive organic molecules that are critical for disease resistance, development of neural tissues, reproduction and optimum somatic growth are not well understood for freshwater consumers.

Connectivity between floodplain and riverine habitats has become of profound applied interest as hydrology in the vast majority of rivers globally has been heavily modified (e.g. Grill et al. 2015; Vörösmarty et al. 2010). Many hydrologically-modified rivers are characterized by reduced connectivity to floodplain habitat, with impacts on fisheries, floodplain productivity and ecosystem processes (Poff et al. 1997; Tockner et al. 2010). The use of environmental flows to restore aspects of the hydrologic regime that promote floodplain-river connectivity has become a management focus globally (e.g. Arthington et al. 2018; Hughes and Rood 2003). However effectively managing these linkages requires a more detailed understanding of the movement of energy and critical resources between floodplain and riverine habitats.

Here we use stable isotope signatures of $\delta^{13}C$ and $\delta^{15}N$ to determine primary energy pathways from basal resources to consumers within three habitats of a lowland temperate Australian river: (1) river channels, (2) anabranches and (3) wetlands. We examine fatty acid profiles of basal resources and consumers among the three habitats to investigate patterns of high- and low-quality food resources and compare them to the fatty acid profiles of four consumers. We expect that transported organic matter sources in the water column (i.e. seston) will be an important basal food resource for aquatic consumers (Hladyz et al. 2012). Given fish and invertebrate responses to flooding, we hypothesise that wetland and anabranch habitats will sustain higher concentrations of dissolved organic carbon (DOC – leached from floodplain litter) and planktonic algae and within the water column and that, as a result, wetland and anabranch seston will support a higher concentration of essential fatty acids for consumers than seston in the river channel.

Methods

Sites

Sampling was conducted on the Ovens River floodplain near Peechelba in north east Victoria, Australia (Figure 1). The Ovens is a relatively unregulated river (with two small reservoirs in its 7780-km^2 catchment – Lake Buffalo and Lake William Hovell), and approximately only 3 % of its water is diverted for irrigation. As such, the flow regime is relatively natural and the floodplain at Peechelba is usually inundated annually in spring by natural flow spates, although floodplain inundation can occur at any time of year following significant rain events in the upper catchment. During high flows, the river is connected to the floodplain by a network of anabranches which inundate dry sections of the floodplain and connect (or fill) numerous temporary and semi–permanent wetlands. During low-flow periods in summer and autumn, anabranches and wetlands accumulate dried leaf litter and large woody debris, and thus facilitate ready exchange of terrestrial organic matter from the floodplain to the main river channel when hydrologic connection is reinstated (Hladyz et al. 2012). Terrestrial litter to aquatic ecosystems is supplied by a dense overstorey of river red gum *Eucalyptus camaldulensis* Dehnh., with stands of river bottlebrush *Callistemon sieberi* DC. adjacent to waterways. The understorey is also densely covered by a mixture of exotic and native grasses, with slender knotweed *Persicaria decipiens* (R.Br.) K.L. Wilson. and common reed *Phragmites australis* (Cav.) Trin. ex Steud. dominant along the margins of waterways.

Food webs were sampled from three habitat types (i.e. river channel, anabranches and permanent wetlands) in the austral summer of 2017 (Figure 1, Table S1). Samples were collected from three replicate sites of each habitat type (9 sites in total), approximately 8 weeks after a floodplain inundation event such that, at the time of sampling, wetlands had been disconnected from anabranches and the river channel for approximately 7 weeks and anabranches had been disconnected from the river channel for approximately 4 weeks.

Sample preparation and analyses

We measured water quality at three randomly-selected locations within each site. We collected all water-quality samples between 1000 and 1400 h to minimize temporal confounding of spatial patterns. We recorded temperature, conductivity, pH, dissolved O_2,

and turbidity at each location with a Hydrolab Quanta multiprobe (OTT Messtechnik, Kempten, Germany). We also collected water samples from each location to measure dissolved organic carbon (DOC) and chlorophyll–*a* (Chl–*a*) (see Appendix S1 for analysis of water samples).

Terrestrial and benthic litter, macrophytes, biofilm, periphyton, seston, benthic and pelagic zooplankton, the predatory waterbug *Anisops thienemanni* and the invasive mosquitofish *Gambusia holbrooki* were collected for carbon and nitrogen stable isotope analysis (SIA) (Hladyz et al. 2012) and fatty acid profiles (FAP) (Conlan et al. 2017). For litter, macrophytes, biofilm and periphyton, samples were collected, dried and ground to a fine powder prior to analysis. For seston, three replicate 250-ml water samples were collected and filtered (53 μm to remove zooplankton) before storage below 4 °C. In the laboratory, a known volume of the samples was filtered onto pre-weighed, pre-ashed quartz filters, prior to drying at 40 °C until constant weight. Zooplankton were sampled using a 12-volt submersible in-line pump with a pumping capacity of 25 L min^{-1} (Nielsen et al. 2008) before immediate freezing (-20 °C). Benthic zooplankton were collected by moving the pump intake approximately 5 cm above the benthos, and pelagic zooplankton were collected from the water column approximately 10-30 cm below the water surface. Zooplankton were later manually removed from bulk samples in the laboratory. *Anisops thienemanni* and *G. holbrooki* were collected from the littoral zone by sweep netting (250-μm mesh sweep net with a D-opening of 300 x 300 mm). Whole benthic and pelagic invertebrates and muscle from *G. holbrooki* were dried and ground to a fine powder prior to analyses.

For SIA, all samples (excluding terrestrial litter and zooplankton) were weighed into tin-foil capsules and combusted in a Europa ANCA-GSL mass spectrometer fitted with a 20-20 IRMS stable isotope analyser without acidification (Europa Ltd., Crewe, U.K.). All isotope values are given in per mil (‰), and standard δ notation is used to describe the relative difference in isotope ratio between the samples and a known standard (Vienna Pee Dee belemnite). For FAP methods followed those used by 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 4-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.

Statistical analysis

All univariate and multivariate models had the same general form. We used 2-factor analyses (fixed factors: habitat and basal resource/consumer) primarily to identify differences in isotopic signatures and fatty acid profiles of basal resources and consumers among habitats. We used three habitat levels (river channel, anabranch and wetland) and levels for basal resources and consumers varied for isotopic and fatty acid analyses. For isotopic analyses, we used five source levels and two consumer levels and, for fatty acid profiles, we used six source levels and four consumer levels (i.e terrestrial litter and pelagic and benthic zooplankton were included for fatty acid analyses). We used permutational analysis of variance (PERMANOVA) in PRIMER v7 (Plymouth Marine Laboratories, 2015) to test for differences in all univariate and multivariate response variables among treatments. All fatty acid percentage data was arcsine–square–root– transformed to meet the assumption of a normal distribution before analyses.

We estimated the relative importance of the various trophic sources to the diets of *A. thienemanni* and *G. holbrooki* using mixing models implemented in the SIAR package in R (Parnell et al. 2008; Parnell et al. 2010). These models take a Bayesian approach to estimate the probability distributions of a consumer diet given the $\delta^{13}C$ and $\delta^{15}N$ signature of each consumer, that of each source (mean ± standard deviation) and the trophic enrichment factor (TEF). We used the TEF values reported by Hladyz et al. (2012), i.e. 0.4 ‰ for $\delta^{13}C$ and 2.3 ‰ for $\delta^{15}N$.

We ran the mixing models for *A. thienemanni* and *G. holbrooki*, including all basal resources that were collected from our habitats. In these models, if two basal resources are located in the same isotopic space, it may not be possible for the model to determine differences in their contributions (Parnell et al. 2013; Ward et al. 2011). To account for that, the models were checked for correlations among resources (by using the function 'siarmatrixplot' of the R package SIAR) and if the resources showed <70 negative correlations, they were deemed distinct (Parnell et al. 2013).

For FAPs we aggregated fatty acids into eight classes; saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated essential fatty acids (LIN, ALA, ARA, EPA and DHA) and bacterial fatty acids (BAFA). BAFA included 10:0 2-OH, 15:0, 17:0 and their branched iso– and anteiso–homologues and 18:1ω7; these fatty acid groups representing essential fatty acids and important FA functional groups (Baek et al. 2017; Guo et al. 2018). Fatty acid functional groups for sources and consumers were analysed together with principal component analysis (PCA) using PRIMER to visualize differences in fatty acid profiles (version 7; Anderson 2005). We used univariate PERMANOVA to analyse trends in isotopic signatures of $\delta^{13}C$ and $\delta^{15}N$ and for comparisons of individual fatty acids among habitats. For all univariate analyses, we carried out tests of significance with the randomization routines in PERMANOVA, after estimating Euclidean distances among samples (Anderson et al. 2008). For all significance tests— both univariate and multivariate—we estimated Monte-Carlo p-values because of the relatively low number of possible permutations for tests of certain model terms (Anderson et al. 2008). We used SIMPER (Clarke 1993) to measure similarity of whole fatty acid profiles among sources and consumers and to compare dissimilarity among habitats.

Results

Water Quality

All measures of water quality, DOC and Chl–*a* differed significantly among our three habitats ($P < 0.05$, PERMANOVA). Mean water temperature was highest in wetlands (25.4 ± 0.3 °C) and lowest in anabranches (22.8 ± 0.5 °C). Mean electrical conductivity was similar between the river channel and wetlands (0.07 ± 0.0 and 0.12 ± 0.0 mScm^{-1}, respectively), but was elevated within anabranches (0.49 ± 0.2 mScm^{-1}). Mean pH values between habitats were similar, ranging between 6.5 ± 0.1 and 6.7 ± 0.1. Mean dissolved O_2 and turbidity followed similar trajectories and were lowest in wetlands (2.1 ± 0.6 mgL^{-1} and 10.2 ± 1.8 NTU,

respectively) and highest in the river channel (6.6 ± 0.0 mgL^{-1} and 19.6 ± 1.7 NTU, respectively). Mean concentration of DOC was highest in wetlands (17.2 ± 6.1 mg CL^{-1}), intermediate in anabranches (8.2 ± 1.0 mg CL^{-1}) and lowest in the river channel (4.7 ± 0.2 mg CL^{-1}). Mean Chl–*a* concentration paralleled DOC, with values ranging from 0.6 ± 0.1 µgL^{-1} in wetlands, 0.5 ± 0.0 µgL^{-1} in anabranches and 0.2 ± 0.0 µgL^{-1} in the river channel.

Stable Isotopes

Delta-^{15}N values for both sources and consumers differed among habitats and sources (P = 0.005, $Pseudo - F_{2,39}$ = 6.78 and P = 0.001, $Pseudo - F_{4,39}$ = 9.29 respectively, Table 2). Among sources, mean δ^{15}N were lowest in wetland benthic litter (1.36 ± 0.43 ‰) and ranged from 3.53 ± 0.93 ‰ in wetland seston to 6.64 ± 0.32 ‰ in river biofilms (Figure 2). *G. holbrooki* collected from anabranches were the most δ^{15}N-enriched consumers (11.30 ± 0.18 ‰), while lowest values were recorded from wetland *A. thienemanni* (7.65 ± 0.23 ‰). Mean values for δ^{13}C of sources where similar among our three habitats, but differed among sources (P = 0.001, $Pseudo - F_{4,39}$ = 9.79, Table 2). Values for δ^{13}C for all consumers where similar (-31.67 ± 1.20 to -35.11 ± 0.67 ‰). Mean δ^{13}C was most depleted in seston in anabranches (-36.95 ± 0.51 ‰) and wetlands (-35.50 ± 0.57 ‰) and most enriched within wetland macrophytes (-24.40 ± 1.41 ‰). Mean δ^{13}C values for aquatic seston and biofilm displayed the same general pattern among habitats, with samples collected from anabranches most depleted, wetlands intermediate and river channel most enriched (Figure 2).

Four-source SIAR V4 Bayesian mixing models allowed us to estimate percent contribution of our potential diet sources and revealed differences in the relative contribution of food sources for consumers within our three habitats. The models showed that, in wetlands, seston was the most likely primary diet source for both *A. thienemanni* and *G. holbrooki* (0.41–0.58 and 0.22–0.43, 50 % credibility interval (Ci), Figure 3) and biofilm likely had a comparatively higher contribution within the river channel to the diet of *A. thienemanni* (0.25–0.46, 50% Ci). Among all habitat types, litter contributed very little to *A. thienemanni* diet (0.01–0.18, 50% Ci) although, for *G. hoolbrooki*, litter contributed a similar proportion to other sources (0.13–0.34, 50% Ci). The contribution of macrophytes to consumer diets in wetlands was lower than for other habitats (0.01–0.16 compared to 0.07–0.31, 50% Ci).

Fatty acids

A total of 53 individual fatty acids were detected from sources and consumers combined. Among sources, SFA contributed between 29.3 and 68.7 % to total fatty acid profiles. Concentrations of all PUFA combined were highest in macrophytes (44 – 67 %) and in seston (11 – 25 %), while mean concentrations of MUFA among sources were highest in biofilms (29 ± 2 %, Table 3). Benthic and terrestrial litter profiles were dominated by BAFA (Table 3). There was a significant interaction in fatty acid profiles between sources and habitats, with main effects also detected for both factors (Table 2, Figure 4). Pairwise comparisons indicated that fatty acid composition of sources differed between the river channel and wetlands and between wetlands and anabranches, but that fatty acid profiles of sources from river channels and anabranches were similar. Fatty acid profiles of all sources differed from one another (Table 3 and Table 4).

Mean percentage contribution of all PUFA to total fatty acid profiles of sources were similar among habitats except in seston, where concentration was highest in wetlands (21 ± 2 %), intermediate in anabranches (15 ± 1 %) and lowest in the river channel (12 ± 1 %). When the proportion of essential fatty acids within seston were interrogated separately, values for ALA (18:3ω3), ARA (20:4ω6), DHA (22:6ω3) and EPA (20:5ω3) reflected the same general pattern across our three habitat types (Figure 5). Mean percentage of ALA ranged from 3 ± 0 % in the river channel to 6 ± 1 % in wetlands and, although ARA varied substantially among habitats, mean values ranged from 0.2 ± 0 % in the river channel, 1 ± 1 % in anabranches and 2 ± 1 % in wetlands. DHA was not detected in river channel seston but accounted for 0.3 ± 0 % of fatty acids in anabranches and 1 ± 0 % CI in wetlands. Mean percentage contribution of EPA to total fatty acids in seston mirrored patterns displayed by other essential PUFA, ranging from 0.5 ± 0 % in the river channel, 1 ± 0 % in anabranches and 2 ± 1 % in wetlands (Figure 5). Mean concentration of BAFA was also higher in wetland (9 ± 3 %) and anabranch (11 ± 3 %) seston than in the river channel (6 ± 0 %).

Despite differences in fatty acid profiles of sources among habitats, overall fatty acid profiles of consumers were similar among habitats, but differed strongly between consumers (Table 2). The mean proportion of MUFA was higher in *A. thienemanni* and *G. holbrooki* (37 ± 2 and 26 ± 2 %, respectively) than in pelagic and benthic zooplankton (12 ± 0.3 % in each). Mean concentrations of PUFA in consumers showed fairly similar patterns (26 ± 2 and 29 ± 2 %, respectively for *A. thienemanni* and *G. holbrooki* and 11 ± 1 and 13 ± 1 %, respectively for pelagic and benthic zooplankton). In contrast to other consumers, zooplankton contained higher mean proportions of SFA (64 ± 1 for pelagic and 62 ± 1 % for benthic, respectively) and BAFA (15 ± 1 for pelagic and 14 ± 1 % for benthic zooplankton, respectively; Table 3).

Bacterial hydroxy fatty acids that contributed to the BAFA grouping were primarily detected from terrestrial and benthic litter sources. Of the BAFA, 10:0 2-OH contributed 8 % and 9 % to within-source similarity of benthic and terrestrial litter fatty acid profiles, respectively, which then also contributed strongly to within-consumer similarity of pelagic (11 %) and benthic zooplankton (10 %) profiles (Table 4). BAFA 10:0 2-OH was not detected from *A. thienemanni* samples but was found in low concentrations in *G. holbrooki* tissue. Percentage contribution to total fatty acid profiles of the essential fatty acid EPA (20:5ω3) was consistently higher in *A. thienemanni* relative to other consumers across all habitat types, with mean values ranging from 5 ± 1 % in the river channel to 4 ± 1 % in anabranches (Figure 6). In contrast, DHA (22:6ω3) was significantly higher (t = 7.11, P < 0.001) in *G. holbrooki,* with mean proportions ranging from 5 ± 1 % in wetlands to 8 ± 2 % in anabranches (Figure 6). ARA (20:4ω6) was not detected in zooplankton sampled from the river channel or from anabranches but was recorded in very low concentration (0.4 % of total fatty acids) in pelagic zooplankton from one wetland. Mean ALA (18:3ω3) concentration was highest in benthic zooplankton (6 ± 1 %) across consumers and mean concentration of LIN (18:2ω6) was highest in *A. thienemanni* (12 ± 0.2 %). Among consumers ratio of omega-3 to omega-6 fatty acids (ω3:ω6) was highest in benthic zooplankton (3.80 ± 0.56) and lowest in *A. thienemanni* (0.62 ±

0.04). Ratio of DHA to EPA was highest in *G. holbrooki* (5.19 ± 0.66) and lowest in *A. thienemanni* (0.04 ± 0.01) and similar between benthic (0.72 ± 0.21) and pelagic zooplankton (0.54 ± 0.12, Table 3).

Discussion

The importance of floodplain inundation for riverine productivity (e.g. Junk et al. (1989) is a central paradigm of freshwater ecology. Here, we identify a mechanism by which that relationship may exist – inundation of floodplains appears to be influence productivity by providing higher quality food resources than are available in the river channel (Figure 7, Table 5).

Our study showed clear differences in the fatty acid profiles of basal resources available to consumers in floodplain wetlands compared with anabranches and the river channel. Wetland seston contained a higher proportion of polyunsaturated essential fatty acids for zooplankton, a pattern also observed in other systems (Smyntek et al. 2008; Wacker and von Elert 2001), known to be important for development and somatic growth of consumers (Guo et al. 2018; Kainz et al. 2004). In particular, EPA (20:5ω3), DHA (22:6ω3), ARA (20:4 ω6), ALA (18:3ω3) and LIN (18:2ω6) were all found in higher concentrations in wetland and anabranch seston, compared with that in the river channel, likely due to algae such as diatoms and cryptophytes (evidenced by higher Chl– *a* concentrations in these habitats), which are known sources of LC-PUFA (Guo et al. 2017; Taipale et al. 2013). In addition to differences in fatty acids, anabranch and wetland seston were also most depleted in δ^{13}C. This suggests the assimilation of dissolved inorganic carbon (DIC) derived from soil–respired CO_2 in groundwater (Burrows et al. 2018), given the importance of groundwater during periods of hydraulic disconnection in this system (Marren and Woods 2011; Yu et al. 2013). Another possible explanation for depleted δ^{13}C in anabranch and wetland habitats is methanotrophic bacterial activity, however we believe this unlikely for three reasons: (1) none of our consumers were highly δ^{13}C -depleted (e.g. <~-40, Kohzu et al. 2004); (2) we did not detect Type I or Type II methanotroph biomarkers (e.g. 16:1ω8 and 18:1ω8, Bowman et al. 1991); and (3) the Ovens River floodplain anabranches and wetlands are known to be sustained by groundwater during periods of disconnection from the river (Marren and Woods 2011; Yu et al. 2013).

Of our two consumers, *A. thienemanni* collected from wetland habitats displayed the highest reliance on seston based on SIA, supporting previous work on the Ovens River where seston was found to contribute most to invertebrate consumer biomass (Hladyz et al. 2012). Isotopic patterns among biofilms mirrored those of seston, with the most depleted mean δ^{13}C values found in anabranch and wetland habitats. Biofilms were predominantly sampled from leaf litter, and are likely dominated by bacteria (given the higher proportions of MUFA and BAFA than for seston). Isotopic signatures of macrophytes varied substantially, likely reflecting the range of taxa, combinations of carbon fixation mechanisms (e.g. C3 or C4), and differences in growth habits.

Although habitat is known to influence fatty acid profiles of other waterbug families (Notonectidae; (Sushchik et al. 2016), we found similar acid profiles in consumers across habitats, despite differences in fatty acid profiles of basal resources among the same habitats. However, differences in high quality LC-PUFA in wetland seston may result in higher densities of zooplankton in anabranches and wetlands (e.g. Müller-Navarra 1995). Australian temperate floodplain wetlands tend to support far greater zooplankton densities than the associated river channel (Ning et al. 2013; Tan and Shiel 1993) – a pattern that was also evident in our study location (mean benthic zooplankton density: 332 ± 122 in the river channel, 4041 ± 1010 in anabranches and 10 967 ± 3298 animals L^{-1} within wetlands, respectively; A. Price, unpublished). Isotopic signatures of consumers were within the ranges of values reported for the Ovens River (Hladyz et al. (2012) and elsewhere (Reid et al. (2008), although our δ^{13}C values are generally more depleted (e.g. -35.11 ± 0.67 ‰ SE compared to -30 ‰, Hladyz et al. 2012). This may be due to differences in taxonomic resolution (i.e. species versus genus) or because we sampled during low flows, when groundwater contributions to the river channel are relatively higher (Marren and Woods 2011).

EPA and DHA are important for neural development and vision, along with cellular membrane functioning (Arts and Kohler 2009; Parrish 2009). Fatty acid profiles of anabranch and wetland *G. holbrooki* were high in DHA compared to basal resources and other consumers, comparable with other studies of freshwater ecosystems (Guo et al. 2017), suggesting that *G. holbrooki* synthesise DHA from dietary precursors or preferentially retains DHA. DHA comprised *c.* 6.5 % of total fatty acids for *G. holbrooki* (Cyprinodontiformes), appreciably less than values reported for Salmoniformes and Perciformes (*c.* 24 % of total fatty acids), but similar to those for Cypriniformes and Siluriformes (*c.* 5% of total fatty acids; Guo et al. (2017). *Gambusia holbrooki* is known to be a generalist carnivore that feeds both at the surface (Pen and Potter 1991) and within the water column (Blanco et al. 2004). These patterns were reflected in our study where *G. holbrooki* incorporated a broad range of basal sources. Previous work on the same river found that terrestrial litter contributed comparatively more to fish tissue (Hladyz et al. 2012) and supplementation of consumer diets with terrestrial inputs may make clear discrimination of food sources difficult.

Anisops thienemanni was found to contain a high proportion of EPA (~4.5% of total fatty acids) relative to other consumers, also consistent with other studies of *Anisops* spp. (Jardine et al. 2015), (e.g. ~ 10-12%, Bell et al. 1994; Ghioni et al. 1996). EPA is preferentially retained by invertebrates generally, and is essential for development, reproduction and hormone regulation for many invertebrates (Guo et al. 2017; Stanley-Samuelson 1994). In contrast with findings for other aquatic macroinvertebrate families (Notonectidae; (Sushchik et al. 2016), we found no significant differences in fatty acid profiles of *A. thienemanni* among habitats. LIN was also found to be higher within *A. thienemanni* than other consumers (~12 % *c.f.* ~6 % for *G. holbrooki* and <2 % for zooplankton) and double previously reported proportions for Notonectidae (Ghioni et al. (1996) Bell et al. (1994). LIN is a precursor of ARA and is only produced by algae and plants (Sargent et al. 1995). Given the low concentration of LIN within the primary food source of *A. thienemani* (i.e. zooplankton; (Barry 1997; Gilbert and Burns 1999; Ho et al. 2011; Reynolds and Geddes 1984), LIN is likely highly retained within *A. thienemani.*

Similarly, zooplankton had higher concentrations of EPA and DHA than their primary food source, seston (e.g. Burns et al. 2011). This suggests that zooplankton selectively assimilate the most physiologically-important fatty acids by preferential incorporation, and/or selective feeding (Burns et al. 2011). However, for ARA (20:4ω6) the opposite was true, since it was present within all basal

resources except terrestrial litter, but not detected (or in very low concentrations) in benthic and pelagic zooplankton. ARA is a precursor for biologically active eicosanoids that are critical compounds of cell membranes and plays many important roles in facilitating and controlling cellular activities (Harrison 1990). Our findings are unusual, since ARA is known to be the most efficiently retained PUFA in planktonic organisms of oligotrophic lakes (Kainz et al. 2004) and may be a limiting factor for fish recruitment success, as it is known to be essential for early development of marine fish (Izquierdo et al. 2000).

Zooplankton are known to target high-quality phytoplankton for food when available (e.g. Cladocera, Galloway et al. 2014). However, in our study, zooplankton fatty acid profiles from all habitats were consistent with concentrations found in litter and, to a lesser degree, biofilm, suggesting that heterotrophic energy pathways were important. BAFA 10:0 2-OH was traced from benthic litter, to benthic and pelagic zooplankton and detected in lower concentrations within *G. holbrooki*. This compound is a hydroxylated fatty acid commonly associated with freshwater bacteria (Baek et al. 2017; Kim et al. 2016; Santos et al. 2018), but has not previously been widely used as a food web biomarker. These results are also supported by the ratio of ω3:ω6 fatty acids among consumers, which were highest in benthic zooplankton and have previously been used as an indicator of heterotrophy in pelagic food webs (Desvilettes et al. 1997; Jardine et al. 2015; Johansson et al. 2016; Véra et al. 2001).

Our results suggest that while inundation of floodplains is a highly important first step in river management, subsequent reconnection to the river may be critical to: 1/ mobilise high quality food resources to the main channel; and 2/ to afford riverine consumers the opportunity to access high quality resources by moving onto the floodplain. In our study, we found clear differences in the quality of basal resources from the river channel and floodplain habitats after a 4-week disconnection, however, further research is required to understand both the optimum connection/disconnection dynamics, along with optimum flooding duration. Season is also likely to be an important factor when planning environmental flows to maximise food quality, as microbial activity can be temperature and light sensitive. Timing may also be important where taxa have seasonal needs for high-quality food to ensure breeding success (e.g. abundant high-quality food is needed in spring and autumn to support recruitment for temperate Australian fish; (Humphries et al. 1999).

Conclusions

This research shows the importance of determining both the quantity and quality of organic matter fluxes into food webs, and the potential role of targeted environmental flows to re-establish critical energy pathways in riverine ecosystems. There is strong evidence of the role of floodplains in generating high-quality food resources critical to animal growth. As a consequence, flow management should seek to ensure that there is flux of these essential materials from the floodplain and into riverine ecosystems.

Acknowledgements

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Figure 1. Location of river channel (RC), anabranch (A) and wetland (W) study sites on the Ovens River floodplain in northeastern Victoria, Australia.
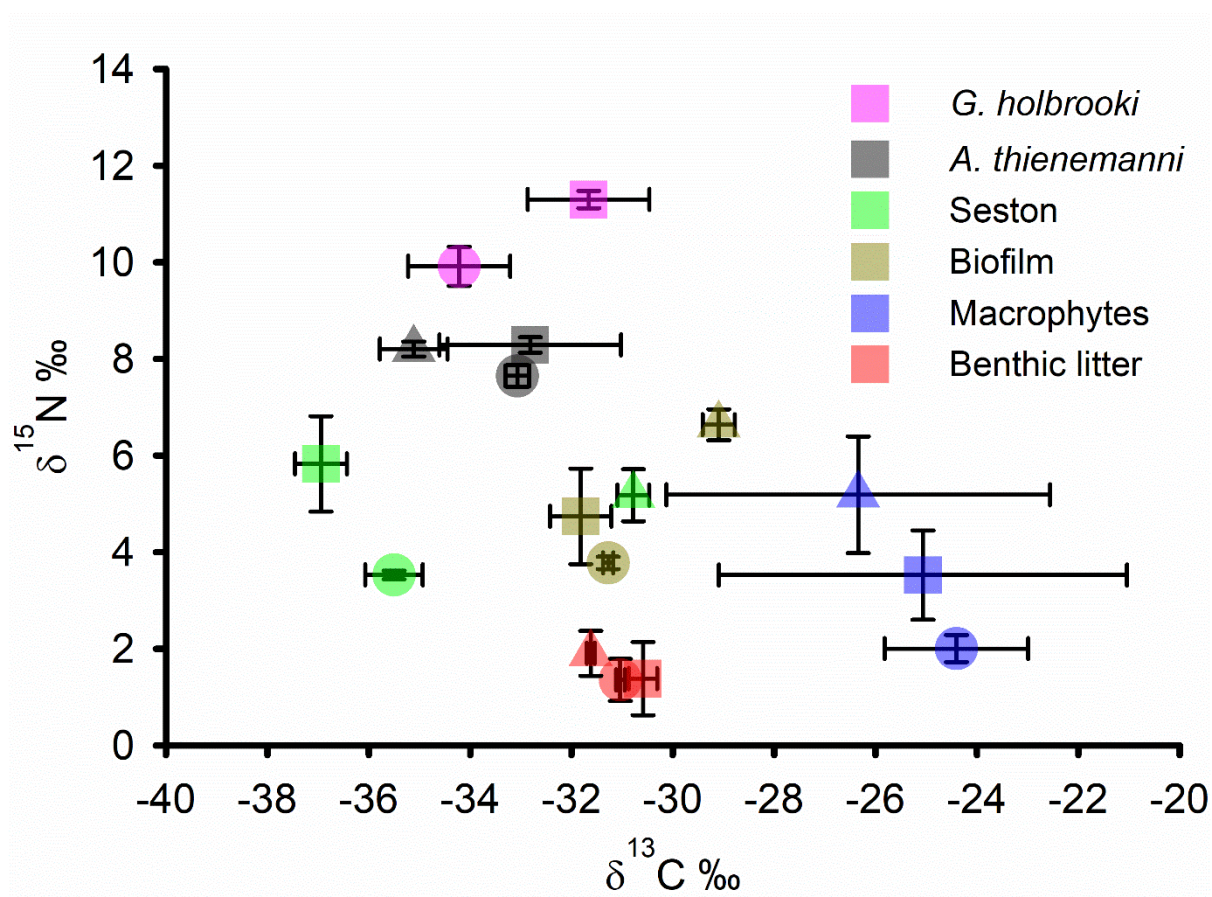
Figure 2. Mean ±SE stable carbon and nitrogen isotopic signatures of sources and consumers from river channel (triangles), anabranches (squares) and wetlands (circles).
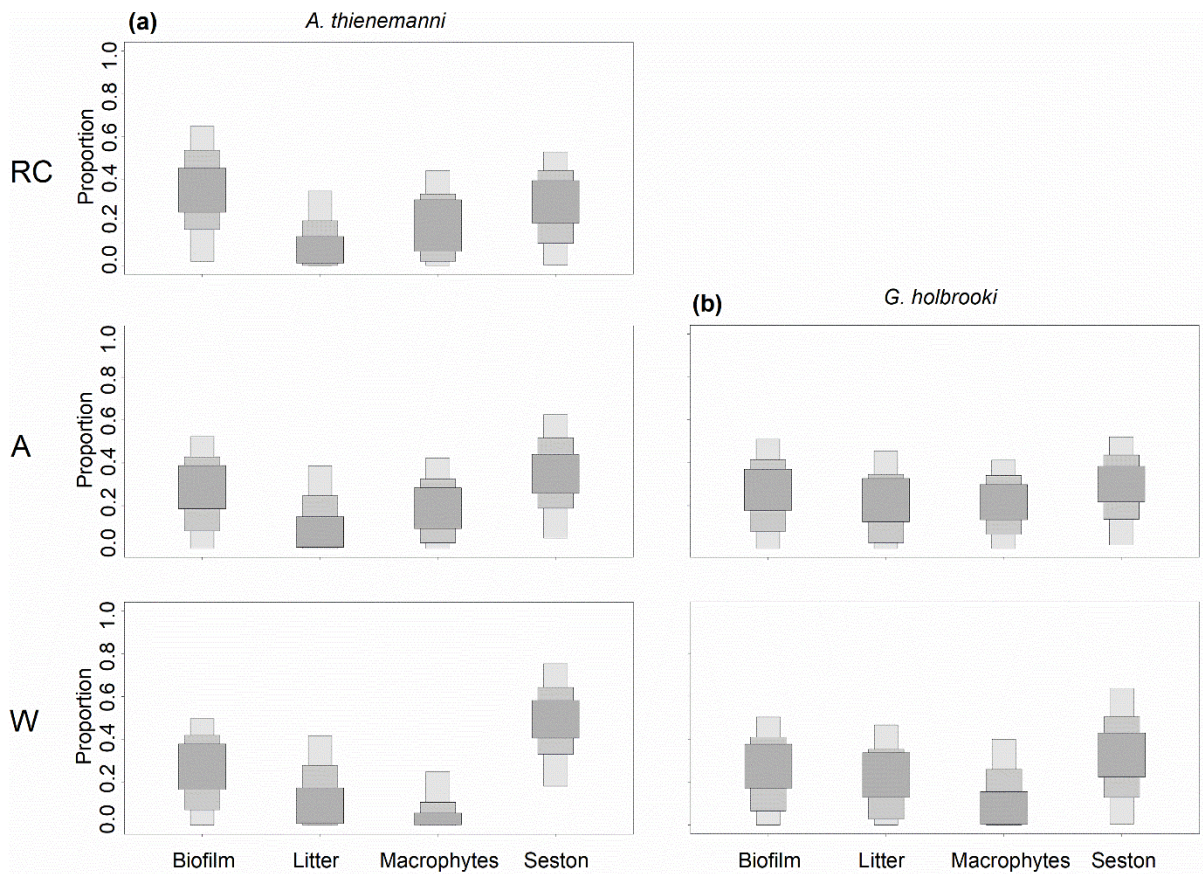
Figure 3. Diet compositions for (a) *A. thienemanni* and (b) *G. holbrooki* estimated by Stable Isotope Analysis in R (SIAR) using four sources (with 50, 75 and 95 % credibility interval (Ci)) from river channel (RC), anabranches (A) and wetlands (W).
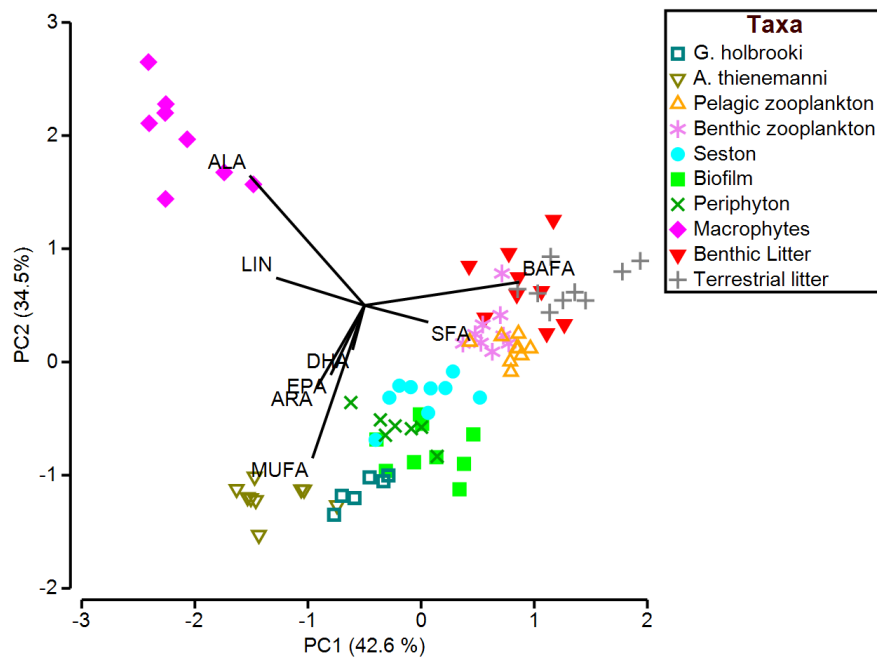
Figure 4. PCA on fatty acid samples of sources and consumers from all habitats combined (arcsine–square–root–transformation on fatty acid % data), saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated essential fatty acids (LIN, ALA, ARA, EPA and DHA) and bacterial fatty acids (BAFA).
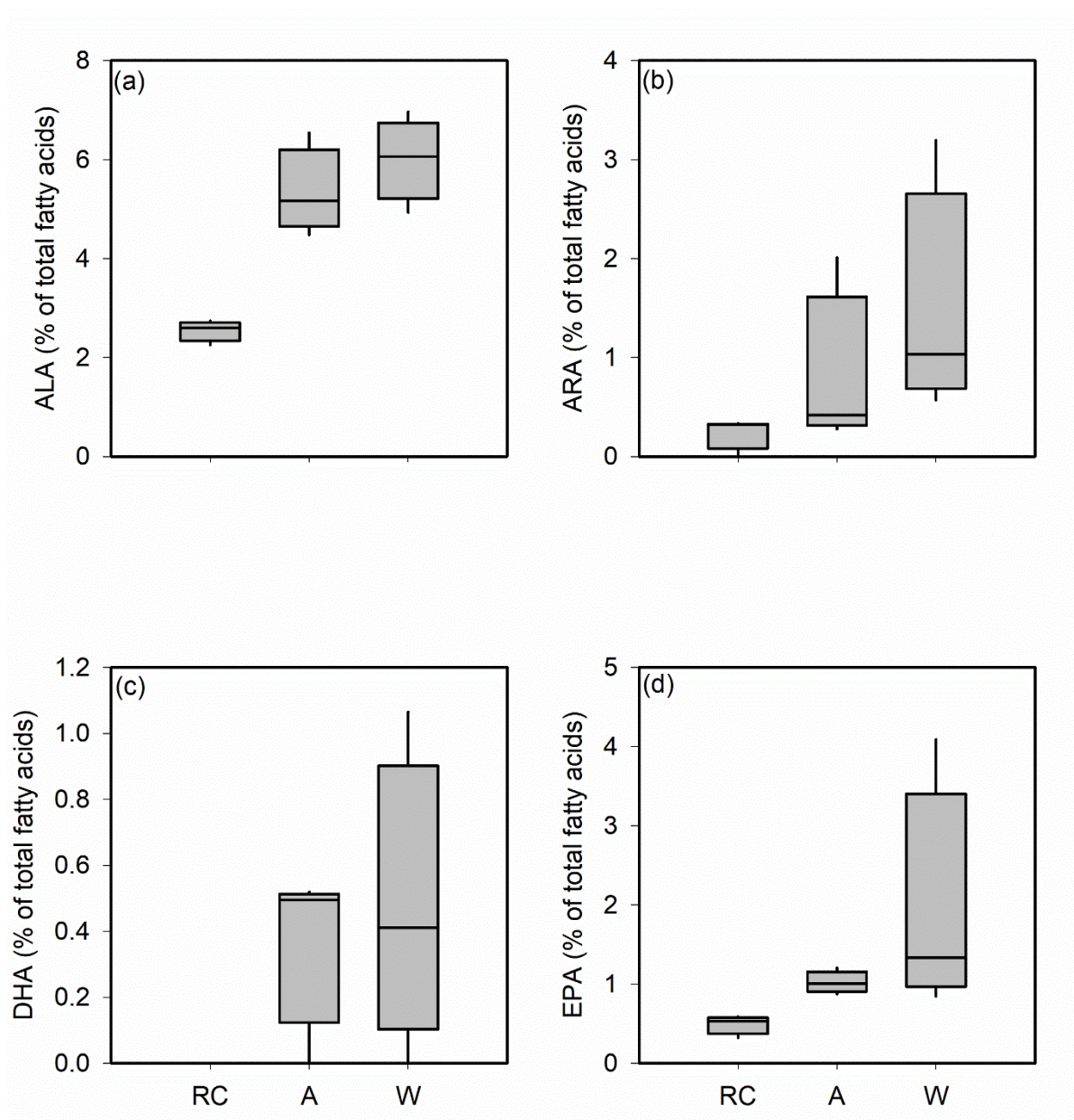
Figure 5. Box plots of percentage contribution of essential fatty acids (a) ALA (18:3ω3), (b) ARA (20:4ω6), (c) DHA (22:6ω3) and (d) EPA (20:5ω3) to seston profiles in river channel (RC), anabranches (A) and wetlands (W).
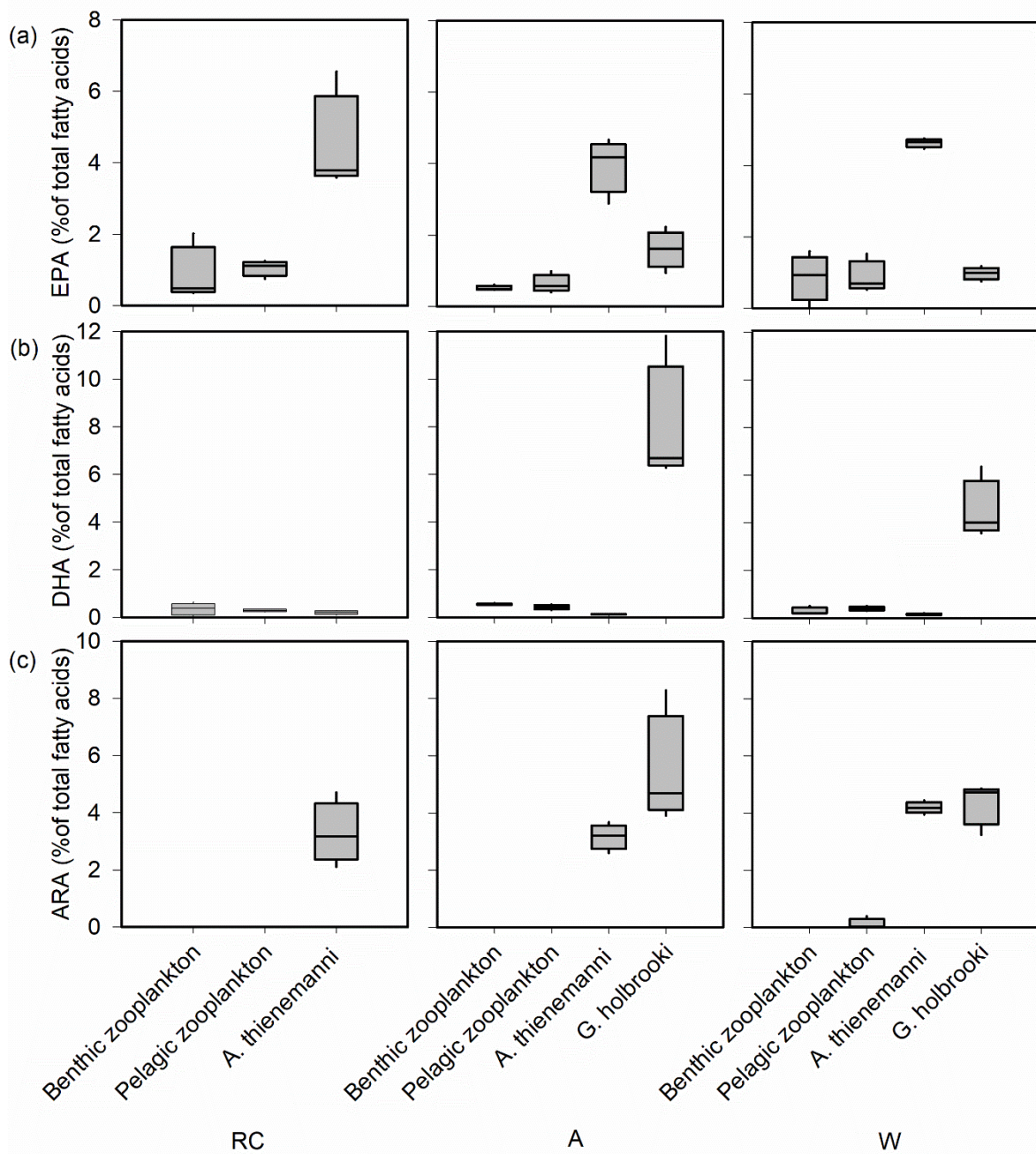
Figure 6. Box plots of percentage contribution of essential fatty acids (a) EPA (20:5ω3), (b) DHA (22:6ω3) and (c) ARA (20:4ω6) of consumer profiles among river channel (RC), anabranches (A) and wetlands (W).

Figure 7. Conceptual diagram summarizing mechanisms by which floodplains may influence essential nutrient dynamics in riverine food webs: 1. Initial flood pulse and floodplain connectivity – liberation of DOC from litter and nutrients from floodplain sediments 2. Disconnection – DOC and nutrients drive microbial activity on floodplain, including synthesis of essential nutrients by algae and bacteria 3. Reconnection – Consumer access to high quality basal resources and transport of high-quality food back to river.

Table 1. Summary of fatty acid sources and function

Fatty acid	Provided by…	Essential for…	References
Long-chain polyunsaturated fatty acids (LC-PUFA, including EPA; 20:5ω3), docosahexaenoic acid (DHA; 22:6ω3), arachidonic acid (ARA; 20:4 ω6), α-linolenic acid (ALA; 18:3ω3) and linoleic acid (LIN; 18:2ω6))	Algae, especially diatoms and cyptophytes	Zooplankton	(Guo et al. 2017; Taipale et al. 2013) ; (Smyntek et al. 2008; Wacker and von Elert 2001)
EPA and DHA	Algae, especially diatoms and cyptophytes	Neural development and vision functions, along with cellular membrane functioning	(Arts and Kohler 2009; Parrish 2009)
BAFA	Bacteria processing Dissolved Organic Carbon		(e.g. Boon 1991)
LIN	Algae and plants	Zooplankton, as a precursor of ARA	(Sargent et al. 1995)
ARA	All basal resources except terrestrial litter, or synthesised by zooplankton from LIN	Cell membranes and cellular activities; fish early development and recruitment success	(Harrison 1990); (Izquierdo et al. 2000)

Table 2. Permutational analysis of variance (PERMANOVA) results for δ^{13}C, δ^{15}N and fatty acid profiles.

Variable	Factor	δ^{13}C			δ^{15}N		Fatty acids		
		df	Pseudo-F	P	Pseudo-F	P	df	Pseudo-F	P
Sources	Habitat	2, 39	0.76	0.497	6.78	0.005	2, 50	3.17	0.009
	Source	4, 39	9.79	0.001	9.29	0.001	5, 50	35.88	0.001
	Habitat x Source	7, 39	1.03	0.448	0.76	0.626	10, 50	1.68	0.031
Consumers	Habitat	2, 14	1.38	0.307	5.95	0.029	2, 32	1.26	0.277
	Consumer	1, 14	0.01	0.998	78.26	0.001	3, 32	61.51	0.001
	Habitat x Consumer	1, 14	0.71	0.429	1.54	0.240	5, 32	1.03	0.427

Table 3. Contribution of fatty acid classes (%) to source and consumer profiles and ω3:ω6 and DHA:EPA ratios for all habitats combined.

	SFA		MUFA		EPA		DHA		ARA		ALA		LIN		BAFA		ω3:ω6		DHA:EPA	
	Mean	±SE	Mean	±SE	Mean	±SE	Mean	±SE	Mean	±SE	Mean	±SE	Mean	±SE	Mean	±SE	Mean	±SE	Mean	±SE
G. holbrooki	42.0	1.3	26.2	2.0	1.3	0.2	6.5	1.2	5.0	0.7	3.0	0.3	6.5	0.4	9.6	1.0	0.9	0.1	5.2	0.7
A. thienemanni	37.4	1.9	36.7	1.6	4.4	0.3	0.2	0.0	3.6	0.3	4.6	0.3	12.0	1.2	4.9	0.5	0.6	0.0	0.0	0.0
Pelagic zooplankton	64.0	0.6	11.8	0.3	0.9	0.1	0.4	0.0	0.0	0.0	3.8	0.3	1.4	0.1	15.3	0.7	2.5	0.2	0.5	0.1
Benthic zooplankton	62.5	1.3	12.3	0.5	0.8	0.2	0.4	0.1	0.0	0.0	6.1	0.5	1.4	0.1	14.5	0.6	3.8	0.6	0.7	0.2
Seston	62.2	1.7	18.7	0.8	1.2	0.4	0.3	0.1	0.9	0.4	4.6	0.6	3.2	0.3	8.8	1.2	1.4	0.2	0.2	0.1
Periphyton	57.8	1.2	25.7	1.4	0.8	0.1	0.1	0.0	0.7	0.1	4.1	0.6	3.7	0.3	7.2	1.2	1.2	0.2	0.1	0.0
Biofilm	52.3	1.2	29.3	1.9	1.1	0.2	0.4	0.1	0.7	0.1	3.1	0.5	3.8	0.4	12.4	1.6	1.3	0.1	0.3	0.1
Macrophytes	38.1	3.4	9.0	1.6	0.2	0.1	0.1	0.1	0.3	0.2	34.1	4.4	14.6	1.5	2.5	0.8	2.1	0.2	0.3	0.2
Benthic Litter	49.2	2.3	10.2	0.9	0.1	0.1	0.0	0.0	0.1	0.1	5.1	0.9	5.1	0.5	26.2	2.5	0.9	0.1	0.0	0.0
Terrestrial litter	46.7	0.8	8.1	0.7	0.0	0.0	0.0	0.0	0.0	0.0	2.4	0.5	6.0	0.7	32.8	2.0	0.4	0.1	0.0	0.0

Table 4. Results of similarity percentage analysis (SIMPER) of fatty acid data for sources and consumers; data not transformed prior to analysis.

Source	FA	Average abundance	Contribution to similarity (%)	Consumer	FA	Average abundance	Contribution to similarity (%)
Seston	16:0	29.9	33.5	G. holbrooki	16:0	22.9	25.2
(85% average similarity)	18:0	9.6	10.3	(88% average similarity)	18:1ω9	16.3	16.2
	18:1ω9	8.4	7.9		18:0	9.6	10.6
	14:0	6.3	6.7		18:2ω6 (LA)	6.5	6.7
	18:3ω3 (ALA)	4.6	4.8		22:6ω3 (DHA)	6.5	5.8
	16:1ω7	4.1	4.2		20:4ω6 (ARA)	5.0	4.5
	18:2ω6 (LA)	3.2	3.4		18:1ω7	3.5	3.7
Periphyton	16:0	34.8	38.5	A. thienemanni	18:1ω9	27.9	28.4
(81% average similarity)	18:1ω9	8.6	8.4	(87% average similarity)	16:0	25.9	27.4
	16:1ω7	9.0	8.1		LA	12.0	11.4
	18:0	6.8	7.4		18:0	6.2	6.4
	14:0	5.7	6.3	Pelagic zooplankton	16:0	24.9	26.4
	18:2ω6 (LA)	3.7	3.8	(90% average similarity)	18:0	17.0	17.6
Biofilm	16:0	29.6	33.4		10:0 2-OH	11.4	11.3
(85% average similarity)	18:1ω9	9.7	9.6		18:1ω9	6.9	7.2
	16:1ω7	9.8	9.2		22:0	5.2	5.2

Source	FA	Average abundance	Contribution to similarity (%)	Consumer	FA	Average abundance	Contribution to similarity (%)
	18:0	7.3	8.0		14:0	4.8	4.8
	18:1ω7	6.5	5.5	Benthic zooplankton	16:0	24.8	26.8
	14:0	4.4	4.3	(88% average similarity)	18:0	16.8	17.7
	18:2ω6 (LA)	3.8	3.8		10:0 2-OH	10.5	10.2
Macrophytes (84% average similarity)	18:3ω3 (ALA)	37.5	36.5		18:1ω9	6.8	7.3
	16:0	25.1	27.0		18:3ω3 (ALA)	6.1	5.9
	18:2ω6 (LA)	15.9	18.0		22:0	5.1	5.3
Benthic litter (81% average similarity)	16:0	21.9	24.6				
	Unknown 1*	15.6	15.9				
	10:0 2-OH	7.6	8.0				
	18:1ω9	6.5	6.6				
	18:2ω6 (LA)	5.1	5.4				
	18:0	4.3	4.8				
	10:0	4.7	4.7				
	18:3ω3	5.1	4.5				
Terrestrial litter	Unknown 1*	22.4	23.1				
	16:0	18.9	20.6				

Source	FA	Average abundance	Contribution to similarity (%)	Consumer	FA	Average abundance	Contribution to similarity (%)
(84% average similarity)	10:0 2-OH	7.9	8.5				
	18:1ω9	6.6	6.3				
	10:0	7.4	6.1				
	18:2ω6 (LA)	6.0	5.6				

*Indeterminate fatty acid thought to be of bacterial origin

Table 5. Summary of key observations and implications

	Observation	Implication
Seston chlorophyll concentrations	Higher in wetlands and anabranches than in the main river channel	Greater production of essential fatty acids in wetlands and anabranches
Essential fatty acids in basal resources	High in wetlands and anabranches, low in the main river channel	Wetlands may be an important source of high-quality food
Essential fatty acids in consumers	Similar across all habitats	Consumers require similar fatty acid profiles to thrive, regardless of basal resource composition
Primary zooplankton food source	Litter and biofilm	Heterotrophic pathways are important
Zooplankton population density	Much higher in wetlands than the main river channel	Zooplankton density in river channels may be limited by availability of fatty acids
Fatty acids in fish *(G. holbrooki)*	Higher than in their primary food source, seston	Selective grazing or selective assimilation allows fish to aggregate fatty acids
$\delta^{13}C$	Depleted in wetlands	Likely assimilation of DIC from groundwater

Appendix S1: Analysis of water samples

All analyses were carried out in the CFE NATA accredited analytical laboratory using the following methods:

Chlorophyll pigments were extracted in hot 90% (v/v) ethanol (5 min at 75 °C) according to standard procedures (ISO 1994). Chlorophyll-a was determined by spectrophotometric absorption and concentrations calculated as µg l^{-1}.

Determinations of DOC were performed by wet oxidation using a model 1010 wet oxidation TOC analyzer by OI Analytical. TIC is determined by measuring the carbon dioxide released when a sample is acidified. The carbon dioxide is purged from the solution and detected by a nondispersive infrared (NDIR) detector that has been calibrated to directly display the mass of carbon dioxide detected. This mass is proportional to the mass of TIC in the sample. After the sample has been acidified and purged of TIC, sodium persulfate is added. This oxidant quickly reacts with organic carbon in the sample at 100°C to form carbon dioxide. The carbon dioxide is purged from the solution and detected by the NDIR. The resulting mass of carbon dioxide is proportional to the mass of DOC in the sample.

Table S1: Location of sites

Site Name	Latitude	Longitude
Wetland 1	36° 9'17.46"S	146°14'5.64"E
Wetland 2	36° 8'49.96"S	146°14'9.06"E
Wetland 3	36° 8'47.90"S	146°14'8.00"E
Anabranch 1	36° 8'32.43"S	146°14'43.93"E
Anabranch 2	36° 8'18.80"S	146°13'50.00"E
Anabranch 3	36° 8'34.30"S	146°13'29.00"E
River channel 1	36° 9'18.66"S	146°14'15.14"E
River channel 2	36° 8'17.00"S	146°14'36.10"E
River channel 3	36° 8'26.90"S	146°14'30.40"E

Appendix 1: Research Activity Reports

Appendix 1.3 Basal resource transfer efficiency between a range of basal resources and to first-order consumers (mesocosm experiments)

1.3.1 The basal food sources for Murray cod (*Maccullochella peelii*) in wetland mesocosms.

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Abstract

The demand for freshwater for human consumption and agriculture over the last century has resulted in many wetland ecosystems being some of the most seriously impacted environs in the world. In attempts to rehabilitate wetland ecosystems environmental flows are used to restore parts of the hydrological regime altered by human water use. The use of environmental water intends to improve ecosystem health, but frequently aims to have specific outcomes for populations of higher-order consumers such as iconic fish and bird species. Research and monitoring mainly focuses on understanding 'flow-ecology' relationships, without investigating the mechanisms underlying them. We sought to understand the importance of different basal food sources to the growth of the endangered Murray cod in temporary wetland systems using fatty acid biomarkers. We flooded replicate mesocosms with two different wetland soils to produce sufficient zooplankton prey to sustain and grow Murray cod larvae for approximately two weeks. The fatty acid profiles of Murray cod and percentages of different biomarkers were compared at the start and finish of the experiment and our results suggest that the most important basal food source is green algae. However, it is unclear if our results can be extended to other wetland systems and we encourage further research both into the relationship between length of wetland flooding and invertebrate densities in other systems. We also encourage research into the mechanistic pathways in which green algae carbon is transferred through food webs to higher order consumers in wetland systems to help generalise our results to other wetlands and support the management of wetlands through the timing and duration of flooding from environmental water.

Introduction

The demand for freshwater for human consumption and agriculture over the last century has resulted in many wetland ecosystems being some of the most seriously impacted environs in the world (Lemly et al. 2000, Millennium Ecosystem Assessment 2005, Vorosmarty et al. 2010). In the Murray–Darling Basin in South-eastern Australia, the ecological impacts of water resource development and the increase in agricultural production have become acute (Kingsford 2000, Jenkins et al. 2005, Murray-Darling Basin Authority 2011b). The level of water use has proved to be unsustainable, both in meeting the demands for irrigated agriculture and domestic supply, and for the ecological health of rivers and floodplain wetlands (Kingsford 2000, Arthington and Pusey 2003, Murray-Darling Basin Authority 2011a, Docker and Robinson 2014). In response, a program of water reform in the Murray-Darling Basin to deliver water to instream channels and wetlands through environmental flows has been developed through the integration of multiple governance layers (Docker and Robinson 2014). Increasing the share of environmental water and improving environmental water management are central elements of this reform (Murray-Darling Basin Authority 2011b, Docker and Robinson 2014).

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). The commonly stated aim of delivering water for the environment is to restore overall ecosystem health, but actual targets are frequently expressed 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 species focused 'flow-ecology' relationships, without necessarily investigating the underlying mechanisms

driving change (Rolls et al. 2017). The production of basal energy and its transfer through food webs is a process fundamental to the success of higher-order consumers (Lowe et al. 2006). However, basal energy sources, their nutritional quality and availability to consumers is likely to vary in time and space across wetland complexes due to differences in local hydrology, vegetation and water quality (Brock et al. 2005, Kelleway et al. 2010). A better understanding of the interaction between hydrological regimes, trophic dynamics and higher-order consumers, would allow a more thorough process-based evaluation of environmental flow programs (Robson et al. 2017).

Nutrition is the supply of materials (food) required by organisms and cells to stay alive, nutrient-dense foods are rich in nutrients relative to their calorie content. The value of a food source to a consumer depends on what materials and compounds the food source has that the consumer cannot synthesise themselves. For example, the freshwater plankter *Daphnia* thrives on a mixed diet of bacteria and algae (Freese and Martin-Creuzburg 2013). Bacteria are thought to provide phosphorus as they have a high phosphorus to carbon ratio, but algae provide polyunsaturated fatty acids and sterols which are important components of tissue membranes and metabolic precursors for many bioactive molecules (Brett and Muller-Navarra 1997, Desvilettes et al. 1997, Vadstein 2000, Martin-Creuzburg et al. 2007). The ratio of basal resource types available to consumers in freshwater environments is influenced by hydrology.

Assessment of the relative importance of basal food sources to higher order consumers and trophic dynamics can be achieved with a variety of methods but the most common in freshwater food-web studies are gut contents analysis (GCA), stable isotope analyses (SIA) and fatty acid profiling (FAP). In many ecosystems, individual food sources have different ratios of $^{13}C:^{12}C$ and $^{15}N:^{14}N$ therefore, food assimilation by animals can be inferred from the isotopic signatures of their tissues (Fry 1991). Fatty acids (FA) are a group of compounds that comprise the majority of lipids found in all organisms. FA profiles can be used to characterize resources consumed by freshwater biota because FAs are not generally degraded and retain their basic form in consumer tissues (Heintz et al. 2004, Budge et al. 2006). Each method has inherent strengths or limitations that affect their suitability for particular investigations (Bromaghin et al. 2017).

Gut content analysis is relatively inexpensive so moderate to large sample sizes can be analysed and taxa in the diet can be identified (Mantel et al. 2004, Li and Dudgeon 2008). However, GCA only provides information on what the animal has eaten over last few meals and differential digestion among taxa may result in biased results. In contrast, SIA integrates diet over time, from days to years depending on the tissue sampled and can be used as an independent verification of trophic level (Perga and Gerdeaux 2005). Some limitations of SIA include that the trophic levels are relative to the carbon source, which must be measured and be distinguishable and the composition and size of prey remain unknown (Bowen and Iverson 2013). SIA also give the overall ratio of the percentage of carbon to nitrogen, which is an indicator of palatability and therefore nutritional value. The higher the ratio of C:N the lower the food quality (Cruz-Rivera and Hay 2000, Southwell et al. 2015).

Similar to SIA, FAP also has the advantage of integrating diet over weeks to months but can only be used to characterize resources consumed by freshwater biota when sources have distinct FA compositions (Traugott et al. 2013). However, FAP are particularly valuable when used to assess the relative quality of food sources. Certain fatty acids are essential for consumer health and in many cases cannot be synthesized *de novo* by the consumer and therefore must be obtained in the diet (Jardine et al. 2015). FAP can identify the presence and relative abundance of essential fatty acids available in the diet. Fatty acid composition of prey is influenced by the nature of basal carbon resources (Brett et al. 2017), which may in turn be influenced by local hydrology. Used together, these methods can provide insight into the origin, transfer and nutritional value of basal resources from primary sources through to higher-order consumers.

Murray cod (*Maccullochella peelii* (Mitchell, 1838)) is the largest exclusively freshwater fish in Australia, its distribution is confined to the Murray-Darling Basin and its conservation status is considered vulnerable by Australian authorities (Australian Society for Fish Biolgy 2016, Department of Environment 2018) and critically endangered by the International Union for Conservation of Nature (Wagner 1996). Due to its conservation status populations of this species are often the target for the delivery of environmental water (Tonkin et al. 2017). However, the relationship between environmental water, basal resources and trophic pathways to support Murray cod populations requires further research. Diet of adult Murray cod consists of

aquatic insects, fish, macrocrustaceans and molluscs (Harris and Rowland 1996, Baumgartner 2007). The natural diet of Murray cod larvae consists mainly of macrotrichid cladocerans and chironomid larvae (Kaminskas and Humphries 2009), while larvae grown in aquaculture mainly consume cladocerans, copepods and chironomid larvae (Ingram & De Silva, 2007). Murray cod larvae hatch in spring, stay near their nesting site for approximately a week, disperse from the nest site by drifting in river currents at night, and continue this behaviour around four to seven days. During this dispersal process, larvae simultaneously absorb the remainder of their yolk sac and begin to feed (King 2002, Humphries 2005). Natural mortality rates are unknown but are approximately 20% in aquaculture situations (McLellan, pers. Comm.). Low mortality, its availability from aquaculture facilities (Allen 1989) and feeding while on egg sac makes this species an ideal candidate for experimentally exploring effects of hydrology on basal food resources and transfer to support fish recruitment food web mesocosm studies.

In this study we tested the following hypotheses in wetland mesocosms:

1. On flooding, the emergence from wetland sediments and subsequent assemblages of invertebrates would differ between wetlands with differing long-term hydrologic regimes.
2. The food value of invertebrates to higher consumers would differ between wetlands.
3. The diet, chemical composition, growth and survival of Murray cod larvae would differ between wetlands due to the differences in basal food resources, availability, chemical composition and nutritional value of prey.

Methods

Study wetlands

The Gwydir wetlands are located in the Gwydir catchment, part of the Murray-Darling Basin in northwest NSW, Australia. The terminal wetlands form part of the Gwydir River (catchment area of 25 900 km^2), which flows from the New England Plateau in the east to the Barwon River at Collarenebri in the west (Environment Climate change and Water NSW 2011). All major tributaries join the Gwydir River upstream of Moree, while downstream the channels form an inland delta of extensive floodplains. The river divides into two floodplains comprising various floodplain vegetation communities, the Gingham Channel to the north and the Gwydir Channel to the south. The principal land use on floodplain and wetlands is private grazing and cropping and the area experiences highly irregular flooding regimes. In all but the largest floods, flows are held in the wetlands and floodplain and do not contribute to flows in the Barwon River.

The Gwydir River system is highly regulated by an extensive series of dams, weirs and diversion channels. Flows into the wetlands are largely regulated by the largest of these, Copeton Dam upstream of Moree. Artificial watering of the wetlands occurs via environmental flows that are released from Copeton. The volumes and timing of the artificial flows is determined by the New South Wales and Commonwealth governments under the direction of the Murray-Darling Basin Authority Basin Plan's environmental watering plan (Murray-Darling Basin Authority 2014).

We chose one site in each of two wetland systems - the Bunnor property on the Gingham watercourse and Old Dromana (OD) property on the Gwydir watercourse (29°16'S 149°21'E and 29°20'S 149°20'E, respectively). The vegetation on the Bunnor and OD sites is dominated by water couch marsh grassland with areas of marsh club rush (Bowen and Simpson 2010, Environment Climate change and Water NSW 2011, Southwell et al. 2015). The similarity in distribution of these vegetation types suggests that the flood frequency is comparable between wetlands. However, since the stabilisation of the Raft, a natural accumulation of felled timber and sediments which effectively dams the river approximately 20km west of Moree, more water is naturally diverted into the Gingham channel (Pietsch 2006, Environment Climate change and Water NSW 2011), in which channel depth and width and estimated bankfull discharge are smaller than on the Gwydir watercourse (Department of Water and Energy 2007). This suggests that in recent times, the Bunnor wetland complex would flood more frequently than the OD wetland, as evidenced by the recent establishment of red gum woodlands in a newly created floodout area in the Gingham watercourse (Environment Climate change and Water NSW 2011).

Mesocosms set up

Dry wetland sediment to a depth of approximately 10 mm was collected from each site. Sediment was put through a 10 mm mechanical sieve to separate soil from roots and vegetation. Twelve 500 L mesocosms were placed in an open-ended poly tunnel covered with white polyethylene which allowed enough ambient light for algae and vegetation growth. The open ends allowed for colonisation of the mesocosms by aerial invertebrates such as Chironomidae. The mesocosms 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 sediment from each wetland was placed in each of six 500 L mesocosms on the third day. The mesocosms had a basal surface area of 0.78 m^2 resulting in a depth of sediment of approximately 10 mm. The mesocosms were gently aerated through the course of the experiment. The mesocosms were allowed to stand for 35 days prior to the introduction of Murray cod larvae (8 days old and sourced from the Department of Primary Industry Fisheries' hatchery at Narrandera), as a pilot study showed that this was likely to produce the diversity and biomass of invertebrates to sustain fish larvae. Individual shield shrimp (*Triops australiensis*) were removed from the mesocosms when observed, as a pilot study had shown that either their activity within the sediment or their feeding behaviour reduced the populations of other invertebrates.

Sampling and processing of biota

Thirty larval point of feeding Murray cod were anesthetised, measured for standard length and weighed prior to deployment of fish to the mesocosms. Fifteen randomly chosen points of feeding Murray cod larvae were introduced to each mesocosm and left for 13 days. Immediately prior to the introduction of Murray cod into the individual tanks planktonic and benthic invertebrates were sampled to provide estimates of their 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 by taking one core (50 mm diameter x 120 mm long with 250 mL volume) for each tank using the method of (King 2004), put through a 250 µm sieve, preserved in ethanol and stained with rose Bengal. On day 13 invertebrate plankton and benthos were again sampled from each mesocosm using the same methods. Additional plankton and benthos were collected using a 400 µm net to provide enough material for stable isotope and fatty acid profiling from each mesocosm prior to them being drained to catch the surviving Murray cod larvae. However, not enough benthic invertebrate tissue material was able to be collected for either stable isotope or fatty acid analyses. Following the draining of the mesocosms, 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).

Captured larvae were anesthetised, measured for standard length, weighed and their entire alimentary canal removed and preserved in ethanol for gut contents analysis. Plankton and benthos samples 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 contents analysis

The contents of the alimentary canal of each fish were spread out on a small petri dish and examined under a binocular microscope. All taxa were counted and identified to the taxonomic level stated above, with the exception of Macrothricidae and Daphniidae, which were pooled as most of them could not be distinguished due to partial digestion of their exoskeletons.

Tissue analyses

C and N content were analysed for fish, zooplankton and the three organic sediment fractions using continuous flow mass spectrometery. Not enough tissue was collected for analysis of benthic fauna. Samples for C:N assessment were prepared by drying at 40°C till constant weight and grinding to a powder that passed through a 500 µm sieve.

Fatty acid profiling followed the methods used by (Conlan et al. 2017). Briefly, lipid was extracted from dry samples soaked in dichloromethane: methanol (CH2Cl2:CH3OH) and quantified gravimetrically on a 4-figure

balance. Fatty acids were extracted following lipid class analysis determined using an Iatroscan MK 6 s 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. The percentage of each species of FA was calculated for each sample and also the total percentages of n-3 and n-6 poly unsaturated fatty acids.

To determine the primary carbon source utilized by Murray cod larvae at the end of the experiment we used the following individual fatty acids 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, Dalsgaard et al. 2003, Alfaro et al. 2006, 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).

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 approaching $1/m$ 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 plankton and benthic invertebrates in each wetland type.

Statistical analyses

The influence of wetland on larval fish length, with mesocosm nested within sediment type, was tested using Permutational Analysis of Variance (PERMANOVA) (Anderson 2001) in the PERMANOVA+ for PRIMER software (Anderson et al. 2008). Similarly, the influence of wetland and zooplankton and benthic invertebrate densities on Murray cod larval survivorship, expressed as a percentage of the 15 larvae alive at the end of 13 days was tested with PERMANOVA. Invertebrate densities for each mesocosm were assumed to be the average of the number of animals collected at the start of the introduction of fish and at the end of 13 days for each habitat type. Euclidean distance was used to form the similarity matrices for analyses. Significant relationships between the main factors and interactions were tested using 9999 randomisations. The taxa responsible for the separation of significantly different groups were tested using similarity percentages (SIMPER) in the PRIMER software. 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. The effect of wetland, time (introduction of fish and end of the experiment) and habitat type (plankton or benthos) on invertebrate assemblage structure was tested using PERMANOVA, with Euclidean distance and 9999 randomisations. Because different methods has been used to collect the plankton and benthos to compare them directly the abundances of each taxon were ranked in descending order within each habitat type and Kendall rank correlation used to form the dissimilarity matrices. Patterns of differences in invertebrate assemblages identified by PERMANOVA were presented diagrammatically using non-metric multidimensional scaling (NMDS) using 50 randomised starts (Clarke 1993).

Results

Invertebrates

A total of 1948 invertebrates from ten taxa were collected in the planktonic and benthic samples. Macrothricidae cladocerans accounted for the majority of animals (53%) followed by Ostracoda (27%),

Chironomidae (9%), Daphniidae (5%) and Chydoridae (3%). The remaining taxa Conchostraca, Harpacticoid, Nematoda, rotifer and snails formed one or less percent of the populations. The ranked abundance of benthic invertebrates differed between wetlands and over time ($F_{1,20}$ = 10.8 and 6.6; p = 0.017 and 0.004, respectively) with no significant interaction. The Bunnor mesocosms had more invertebrates than the OD mesocosms and abundances increased over time (Figure 1). The total abundance of planktonic invertebrates differed between the wetland mesocosms ($F_{1,20}$ = 9.5; p = 0.006) with no significant time effects and no interaction. The Bunnor mesocosms had on average more invertebrates than the OD mesocosms which was consistent over time (Figure 1).
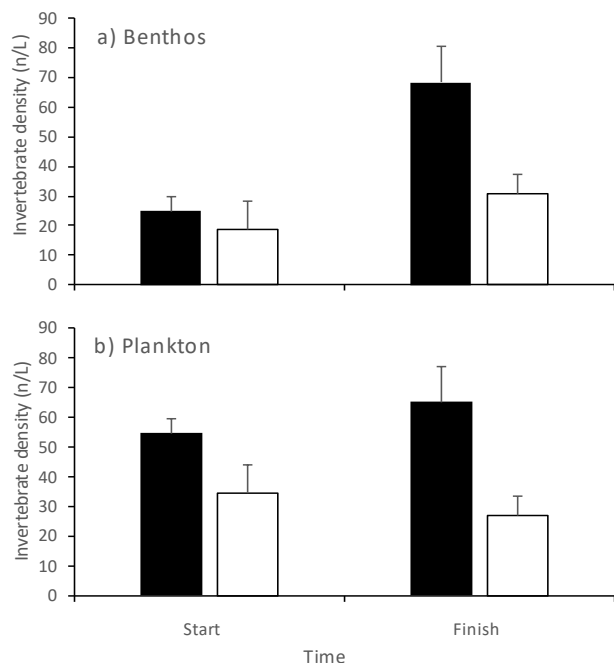

Figure 1. Mean invertebrate densities (± 1 S.E.) in a) benthos and b) plankton. Black columns denote Bunnor mesocosms and white Old dromana

Invertebrate assemblage structure differed between wetlands ($F_{1,40}$ = 9.8; p = 0.001), habitats ($F_{1,40}$ = 8.9; p = 0.001) and there was a significant interaction between habitats and time ($F_{1,40}$ = 3.5; p = 0.004). The other sources of variation in the PERMANOVA model were not significant, suggesting that the differing change over time within habitats (as observed by the habitat by time interaction) was consistent regardless of wetland type. Pairwise tests indicate that there was no significant difference between planktonic and benthic samples at the start of the experiment, but these two habitats differed at the end ($t_{1,20}$ = 3.2; p = 0.001). In addition, the was no significant difference between the plankton assemblages at the start and end of the experiment but the benthos differed between the two sampling occasions ($t_{1,20}$ = 2.1; p = 0.001). This suggests that the significant habitat by time interaction was driven by the benthic assemblage structure becoming more dissimilar to the other habitat and times at the end of the experiment. The Bunnor samples clearly separate from the OD samples in ordination space (Figure 2a). The distance between the benthic samples and the other samples increases at the end of the experiment (Figure 2b).
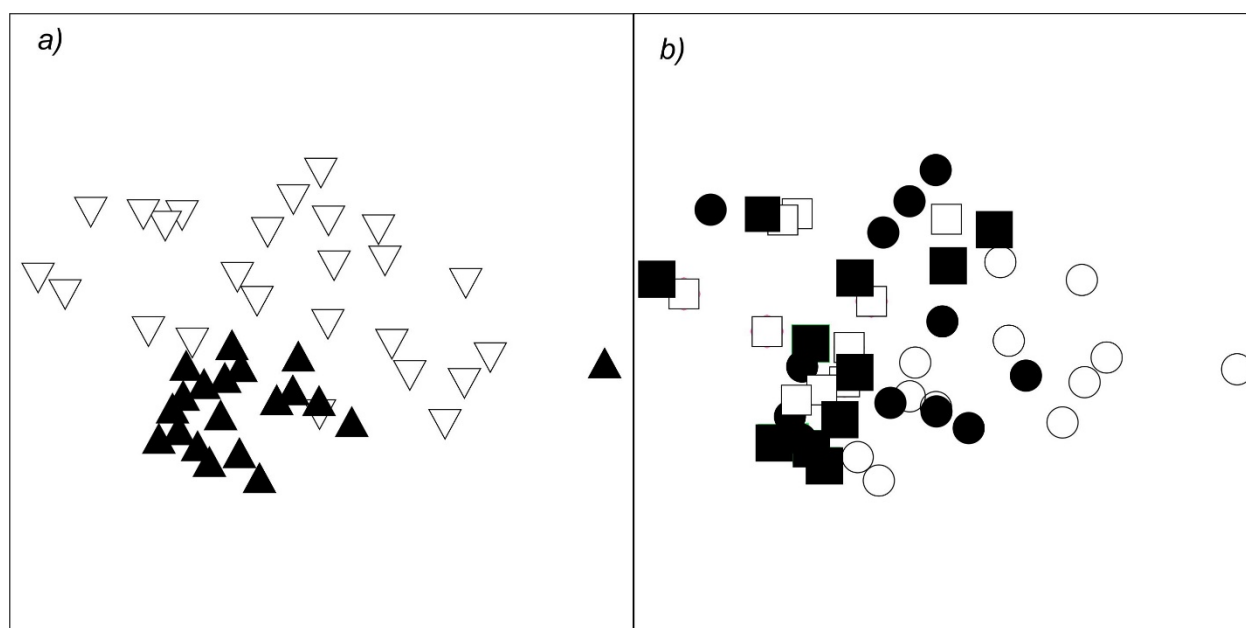
Figure 2. MDS ordination of invertebrate assemblages. a) coded for wetland type (black triangles = Bunnor samples; white triangles = OD samples. B) the same ordination coded for different habitats at the start and end of the experiment (circles = benthos; squares = plankton; black shapes = start; white shapes = end).

Five taxa contributed to the differences between wetlands with Daphniidae, Ostracoda, Chydoridae and Macrothricidae all ranking higher in the Bunnor mesocosms (Table 1). Only Chironomidae ranked higher in the OD mesocosms. Macrothricidae, Nematodes and Chydoridae all ranked lower in the benthos at the end of the experiment compared with Chironomidae, Ostracoda and snails increasing in rank. Daphniidae, Chydoridae, Ostracoda and Macrothricidae all had greater ranks in the plankton compared with the benthos at the end of the experiment. Only Chironomidae ranked more highly in the benthos.

Table 1. Average ranks of taxa contributing to differences between various groups of samples SIMPER

	Average rank		
Wetland samples	Bunnor	Old Dromana	% contribution
Chironomidae	8.5	11.0	14.9
Daphniidae	8.7	7.3	14.2
Ostracod	12.2	10.5	14.0
Chydoridae	8.4	8.4	13.7
Macrothricidae	12.3	12.0	13.1
Benthos samples	Start	End	
Macrothricidae	16.1	6.2	17.5
Chironomidae	13.1	15.8	15.2
Nematode	8.0	7.3	13.4
Ostracod	10.0	10.8	12.0
Chydoridae	9.4	1.0	11.6
Snail	4.1	7.6	10.6

Habitats at end	Average rank		
	Benthos	Plankton	
Daphniidae	5.2	14.9	17.1
Chironomidae	15.8	4.0	17.0
Chydoridae	1.0	13.8	16.4
Ostracod	10.8	12.0	13.2
Macrothricidae	6.2	12.8	12.8

Murray cod larvae

Murray cod larvae introduced to the mesocosms had a mean weight of 7.9 ± 0.2 µg (1 S.E.) and were 10.9 ± 0.1 mm standard length. Over the 13 days they increased in mean weight to 40 ± 0.9 µg and mean length to 14.4 ± 0.1 mm. There was no significant difference in either mean weight or length between the wetlands, however, there was significant differences in both mean weight and length among the OD mesocosm ($F_{1,10}$ = 11.0, p = 0.0001).

Survival of larvae differed between wetland mesocosms ($F_{1,8}$ = 5.6, p = 0.043) with mean survival greater in Bunnor (Figure 3). Survival in both mesocosm types was positively related to the density of zooplankton ($F_{1,8}$ = 10.3, p = 0.011) but not benthic invertebrate density ($F_{1,8}$ = 0.15, p =0.701). There was no significant interaction between mesocosm type and plankton density ($F_{1,8}$ = 0.85, p = 0.407) indicating that the relationship between survival and density was similar between the wetlands.
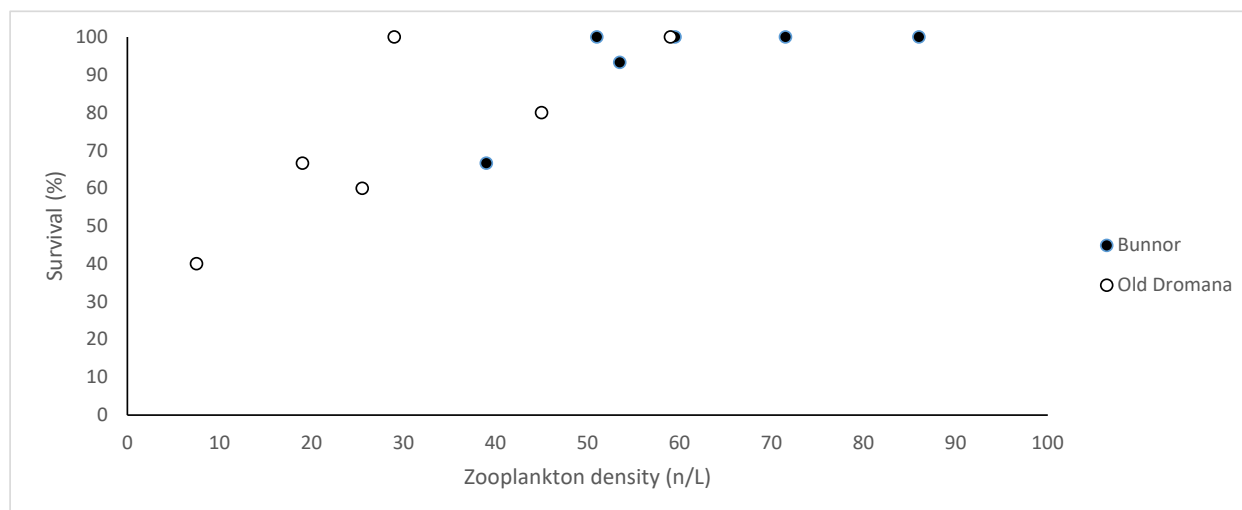

Figure 3. Relationship between zooplankton density and Murray cod larval survival. Black circles Bunnor wetland mesocosms and white circles Old Dromana mesocosms.

A total of 885 individual invertebrates from five taxa were identified from the 135 fish gut samples. The majority were Macrothricidae/Daphniidae cladocerans (60%) followed by Chironomidae (20%), Chydoridae (13%), Ostracoda (5%) and Harpacticoida (2%). Gut contents were significantly different between wetlands ($F_{1,10}$ = 15.8, p = 0.008), SIMPER indicating that Macrothricidae/Daphniidae cladocerans occurred more frequently in the Bunnor fish and Chironomidae occurred more frequently in the OD fish.

There was a significant interaction between taxa and habitat and taxa and wetland for the Manly-Chesson index α ($F_{3,80}$ = 13.8, p = 0.0001 and 4.6, p = 0.005 respectively). Chironomidae appeared to be a preferred dietary item in the plankton and Chydoridae in the benthos in both wetlands (Figure 4). In contrast, Macrothricidae/Daphniidae cladocerans in both habitats of the OD fish and Ostracoda in both wetlands and habitats appeared to be avoided.
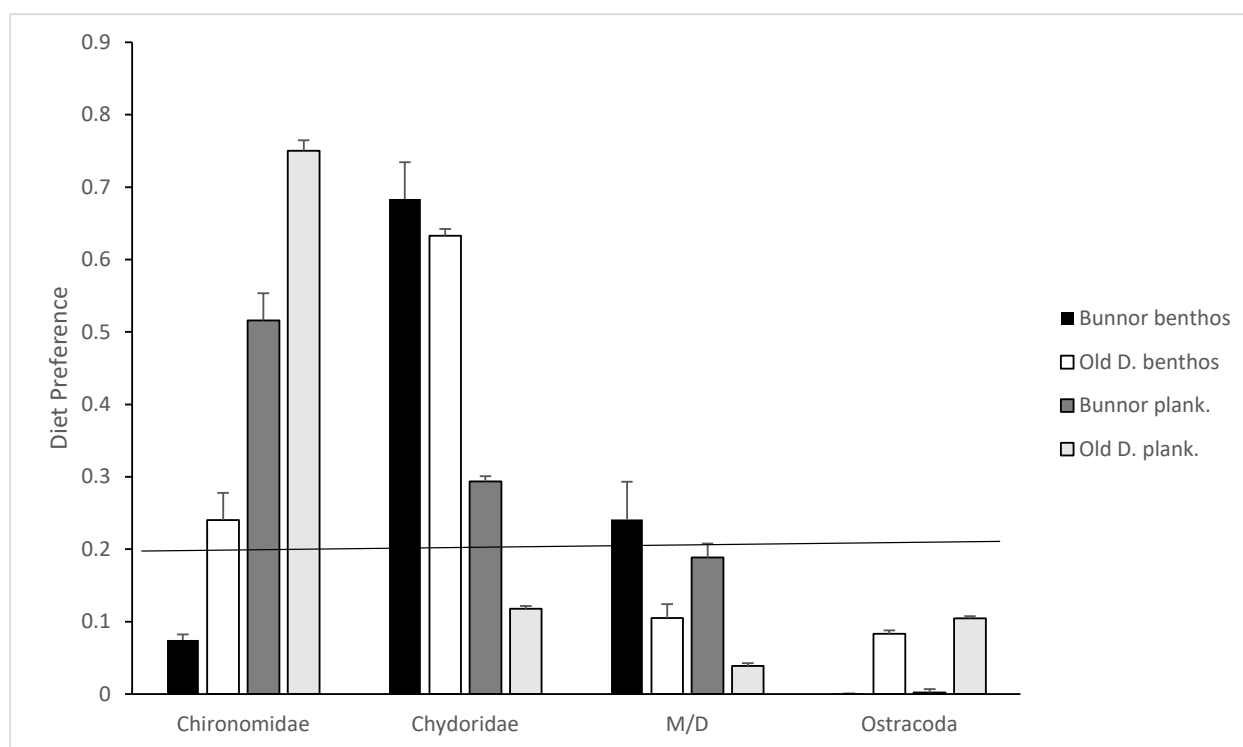
Figure 4. Manly-Chesson prey selection index α, the horizontal line is the neutral value for selectiveness.

Fatty acid profiles

A total of 47 individual fatty acids were identified from the substratum fractions, zooplankton and fish (Table 2). There was a significant difference between the FAP of the different substratum fractions and biota and wetland type and a significant interaction between these factors (F > 3.4, p = 0.0001). Pairwise contrasts indicated that each of the substrate fractions and fish differed between wetlands but the FAP of zooplankton was not significantly different. PERMDISP indicated that the significant difference between substrate fractions and fish between wetlands was most likely due to the variation in FAP between replicates from OD being significantly greater than the Bunnor replicates.

The fatty acids present in greater than 10% in the Murray cod larvae at the start of the experiment included 16:0 (19.5%), 18:1n-9 (17.4%), 18:0 (10.6%), 18:2n-6 (10.6%) and 22:6n-3 (10.3%). The mean percentage of individual FAs in larvae between wetlands and compared with larvae at the start of the experiment was similar with the exception of 18:2n-6 which was absent from larvae at the end of the experiment (Table 2). The fatty acids that were greater than 10% in the zooplankton included 16:0, 18:3n-3, 18:1n-9 and 18:0. The FA greater than 10% in the Bunnor VFPOM and FPOM samples included 16:0 and 18:1n-9 and these two FA were also greater than 10% in the OD samples but so was 22:1 (isomers) as well. There were four FA greater than 10% in the Bunnor CPOM samples including 16:0, 18:1n-9, 18:3n-3 and 16:1n-7. In contrast, 16:0 was the only FA greater than 10% in the OD CPOM samples.

Table 2. Mean percentage (± 1 S.E.) of each fatty acid in substratum fractions and biota in each wetland.

Fatty acid	Bunnor						Old Dromana				
	START FISH	VFPOM	FPOM	CPOM	Zooplankton	Fish	VFPOM	FPOM	CPOM	Zooplankton	Fish
8:0	0	<1	<1	<1	0	0	<1	0	<1	0	0
10:0	0	<1	<1	<1	<1	0	<1	<1	<1	<1	0
11:0	0	<1	0	<1	0	0	1.3 ± 0.5	<1	<1	0	0
12:0	0	<1	<1	<1	<1	<1	1.3 ± 0.4	<1	<1	<1	<1
14:0	<1	3.7 ± 0.2	2.8 ± 0.1	2.7 ± 0.1	1.9 ± 0.6	<1	4.6 ± 0.2	4.9 ± 0.3	4 ± 0.3	3.6 ± 0.5	1.3 ± 0.1
15:0	<1	1 ± 0.2	1 ± 0.1	1.1 ± 0.1	<1	<1	3.5 ± 0.2	4.3 ± 0.2	3.6 ± 0.3	1.6 ± 0.2	<1
16:0	19.5	23.7 ± 0.5	22.5 ± 0.7	21.8 ± 0.7	20.4 ± 2.2	20.9 ± 0.1	20.5 ± 1.1	20.3 ± 0.5	20.8 ± 0.8	23.3 ± 0.6	22 ± 0.5
17:0	1.2	1.1 ± 0.2	1 ± 0.2	1.3 ± 0.2	1.6 ± 0.2	1.5 ± 0	<1	<1	<1	1.2 ± 0.4	1.7 ± 0.1
18:0	10.6	9.2 ± 0.6	7.8 ± 0.7	7.2 ± 0.5	11 ± 2.2	11.5 ± 0.2	6.1 ± 0.5	7.1 ± 0.3	8.2 ± 0.6	8.2 ± 0.6	12.1 ± 0.4
20:0	<1	2.4 ± 0.2	1.8 ± 0.1	1.7 ± 0.2	<1	<1	<1	<1	<1	<1	<1
21:0	<1	1.5 ± 0.5	1.1 ± 0.2	1.2 ± 0.1	1.5 ± 0.6	1.2 ± 0	5.2 ± 2.8	3.6 ± 2.5	5.9 ± 2.9	<1	<1
22:0	<1	0	0	0	<1	0	0	0	0	0	0
24:0	0	3.9 ± 0.2	2.8 ± 0.3	2.6 ± 0.3	<1	<1	6.7 ± 0.3	8.5 ± 0.5	6.8 ± 0.3	<1	<1
14:1n-5	<1	1 ± 0.2	1.2 ± 0.1	1.4 ± 0.2	<1	<1	4 ± 0.3	6 ± 0.5	5.4 ± 0.4	1.7 ± 0.4	<1
15:1n-5	<1	<1	<1	<1	0	<1	<1	<1	<1	<1	<1
16:1n-7	2.2	5 ± 0.7	11.1 ± 1	10.9 ± 1.2	2.6 ± 0.4	2.1 ± 0.2	5.7 ± 1.6	5.3 ± 0.3	5.7 ± 0.9	7.5 ± 1.9	4 ± 0.4
17:1n-7	<1	<1	<1	<1	<1	<1	<1	0	<1	<1	<1
18:1n-7	4.4	2.1 ± 0.2	3.9 ± 0.3	3.2 ± 0.4	6.1 ± 0.7	4.9 ± 0.1	<1	1 ± 0.6	3 ± 0.2	7.7 ± 1	4.4 ± 0.4
18:1n-7 t	<1	0	0	0	0	0	0	<1	0	0	0
18:1n-9	17.4	11.5 ± 0.6	13.8 ± 0.7	15.1 ± 0.6	13.5 ± 0.8	12.3 ± 0.2	11.5 ± 0.5	10.3 ± 0.5	9.4 ± 0.2	14.5 ± 1.7	12.4 ± 0.3
18:1n-9 t	0	0	0	<1	0	<1	<1	0	<1	<1	<1
20:1 (isomers)	<1	4.6 ± 0.8	2.9 ± 0.6	2.2 ± 0.3	3.2 ± 0.6	1.1 ± 0.1	<1	0	<1	1.5 ± 0.4	1.2 ± 0.1
22:1 (isomers)	<1	9.9 ± 1.7	8.2 ± 1.2	5.4 ± 1	3.8 ± 1.2	1.5 ± 0.1	12.6 ± 2.2	13.5 ± 2.5	8.9 ± 2.7	1.7 ± 0.3	1.5 ± 0.2
24:1n-9	<1	<1	<1	<1	<1	<1	0	0	0	0	<1
18:3n-3	1	5.7 ± 0.5	6.8 ± 0.8	11.5 ± 1.7	14.6 ± 3.1	2.8 ± 0.2	4.7 ± 0.6	2.9 ± 0.7	3.8 ± 0.4	12.6 ± 1.3	3.5 ± 0.4
18:4n-3	0	<1	<1	0	0	0	0	0	<1	0	0
20:3n-3	0	0	0	0	0	<1	0	0	0	0	<1

Fatty acid	START FISH	Bunnor					Old Dromana				
		VFPOM	FPOM	CPOM	Zooplankton	Fish	VFPOM	FPOM	CPOM	Zooplankton	Fish
20:5n-3	1	<1	3.4 ± 0.6	2.6 ± 0.7	5.1 ± 1.3	2.5 ± 0.1	<1	0	1.2 ± 0.8	3.7 ± 0.2	2.1 ± 0.2
22:3n-3	0	0	0	0	0	0	0	0	0	0	<1
22:5n-3	2.2	1.2 ± 0.4	<1	<1	<1	3.2 ± 0.1	<1	0	<1	<1	2.3 ± 0.1
22:6n-3	10.3	<1	<1	<1	1.2 ± 0.4	12.9 ± 0.2	0	0	0	0	11.7 ± 0.8
24:5n-3	0	0	0	0	0	<1	0	0	0	0	<1
24:6n-3	<1	0	0	0	0	<1	0	0	0	0	<1
16:2n-4	0	0	<1	<1	0	0	0	0	<1	0	<1
16:3n-4	0	<1	<1	<1	0	0	0	0	<1	0	0
18:3n-4	0	<1	0	0	0	<1	0	0	0	0	<1
18:2n-6	10.6	0	0	<1	2.2 ± 2.2	0	<1	0	<1	0	0
18:2n-6 t	<1	0	0	0	0	<1	<1	0	<1	0	<1
18:3n-6	<1	1.4 ± 1.2	<1	<1	<1	<1	<1	0	<1	<1	<1
20:2n-6	1.1	1.3 ± 0.7	<1	<1	<1	<1	<1	0	<1	<1	<1
20:3n-6	1.3	<1	<1	<1	<1	1.8 ± 0.2	0	2.6 ± 2	<1	<1	1.4 ± 0.2
20:4n-6	7.9	3.1 ± 1.3	2.5 ± 0.5	1.9 ± 0.4	4.4 ± 0.8	10.2 ± 0.1	4.9 ± 0.2	6 ± 0.3	4.1 ± 0.7	5 ± 0.3	8.8 ± 0.4
22:2n-6	<1	<1	<1	<1	0	0	0	0	0	0	0
22:4n-6	1.8	0	0	0	<1	1.7 ± 0	0	0	0	<1	1.5 ± 0.1
22:5n-6	<1	0	<1	0	<1	<1	0	0	<1	0	<1
Unknown 1	0	<1	<1	<1	<1	<1	<1	0	<1	<1	<1
Unknown 2	0	1.5 ± 0.3	<1	<1	<1	0	2.1 ± 1.3	0	2 ± 1.2	<1	0

Potential food source quality

There was a significant difference in the C:N ratio between the various potential food sources and wetlands and a significant interaction between those factors (F = 265, 17 and 18 respectively, p< 0.001). Pairwise tests indicated that the mean C:N ratio of the OD substratum fractions were significantly greater than the Bunnor wetland (t > 0.48, p < 0.01) (Figure 5). The higher ratio indicates that the OD substratum is of lower nutritional value than the Bunnor substratum. In contrast, there was no significant difference in the C:N ratio the zooplankton or Murray cod larvae between wetlands.
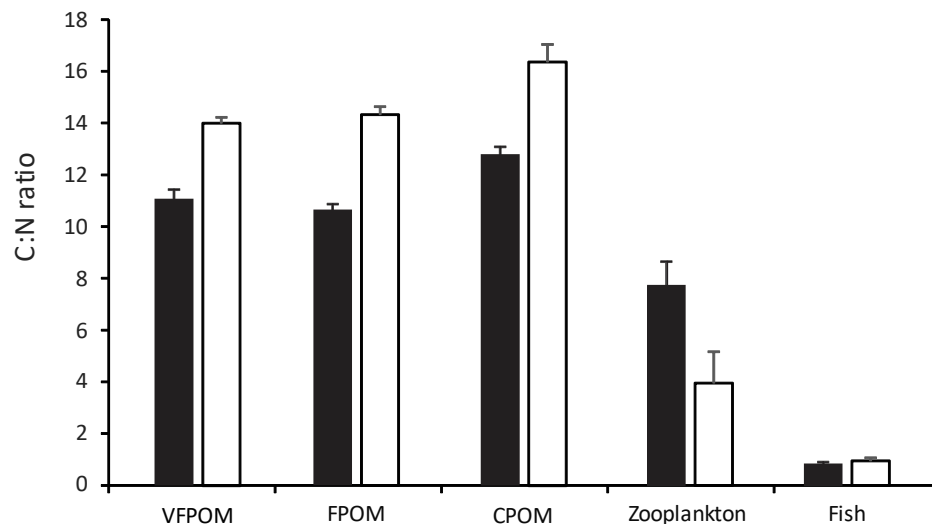

Figure 5. Mean (± 1 S.E.) carbon to nitrogen ratios in various potential food sources. Black columns are from Bunnor mesocosms and white from Old Dromana mesocosms.

There was a significant difference between the mean total n-3 and n-6 PUFA between the various potential food sources and wetlands and no interaction (F = 65 and 20 respectively, p< 0.001). Pairwise tests indicated that the mean total PUFA of the OD substratum fractions were significantly less than the Bunnor wetland (t > 0.49, p < 0.01) (Figure 6). The lower mean total indicates that the OD substratum is of lower nutritional value than the Bunnor substratum. In contrast, there was no significant difference in the mean total PUFA of the zooplankton or Murray cod larvae between wetlands.
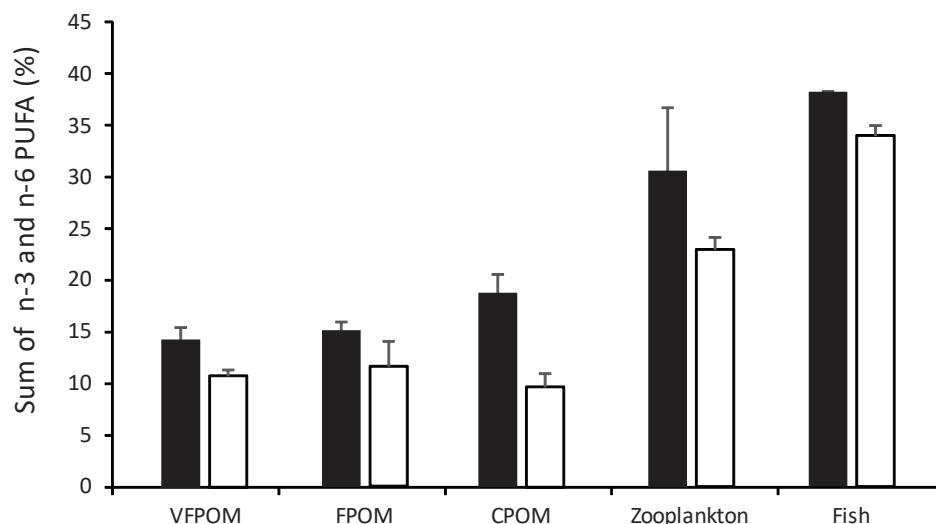
Figure 6. Mean (± 1 S.E.) sum of n-3 and n-6 polyunsaturated fatty acids (PUFA) in various potential food sources. Black columns are from Bunnor mesocosms and white from Old Dromana mesocosms.

Biomarkers

There was a significant difference between the mean percentage of bacterial fatty acid biomarkers between different animal groups and substratum fractions and wetlands and there was a significant interaction between those factors ($F > 3.9$, $p < 0.05$). Pairwise tests indicated that the mean percentage of bacterial fatty acids was significantly greater in all the OD substratum factions and fish ($t > 2.6$, $p < 0.01$) but not for zooplankton (Figure 7).

The mean percentage of the cyanobacterial fatty acid biomarker was significantly different between the invertebrates and fish and substratum fractions and wetlands, and there was a significant interaction between those factors ($F > 3.9$, $p < 0.001$). The mean percentage of the cyanobacterial fatty acid was significantly greater in all the OD substratum factions and fish ($t > 2.5$, $p < 0.01$) compared with the Bunnor wetland but there was no significant difference in the percentages for zooplankton between wetlands, even though the mean percentage is almost double in the OD wetland (Figure 7).

There was a significant difference between the mean percentage of the diatom fatty acid biomarker between different animal groups and substratum fractions and wetlands ($F = 11.1$ and 18.4 respectively, $p < 0.002$) and with no significant interaction between those factors. The mean percentage of the biomarker was not significantly different between CPOM, fish and FPOM but was significantly different between all the other groups. In general, zooplankton had the greatest percentage of the biomarker and VFPOM the least (Figure 9). The Bunnor substratum fractions, and animal groups all had a significantly greater percentage than the OD groups.

The mean percentage of the green algae fatty acid biomarker was significantly different between the different animal groups and substratum fractions and wetlands and there was a significant interaction between those factors ($F > 4.5$, $p < 0.05$). Pairwise tests indicated that the mean percentage of the algal indicator was significantly greater in all the Bunnor CPOM and FPOM ($t > 3.4$, $p < 0.001$) but there was no significant difference the percentages for VFPOM, zooplankton or fish between wetlands (Figure 7).

There was a significant difference between the mean percentage of the fungal bacterial fatty acid biomarker between different animal groups and substratum fractions and wetlands and there was a significant interaction between those factors ($F > 3.5$, $p < 0.05$). The mean percentage of the fungal indicator was significantly greater in all the Bunnor CPOM and FPOM ($t > 3.4$, $p < 0.001$) but there

was no significant difference in the percentages for VFPOM, zooplankton or fish between wetlands (Figure 7).

The mean percentage of each fatty acid biomarker increased in the fish from the start to the end of the experiment with the exception of the fungal indicator (Figure 7). The greatest percentage increase was for green algae (308%) followed by diatoms (230%), cyanobacteria (204%) and bacteria (140%).
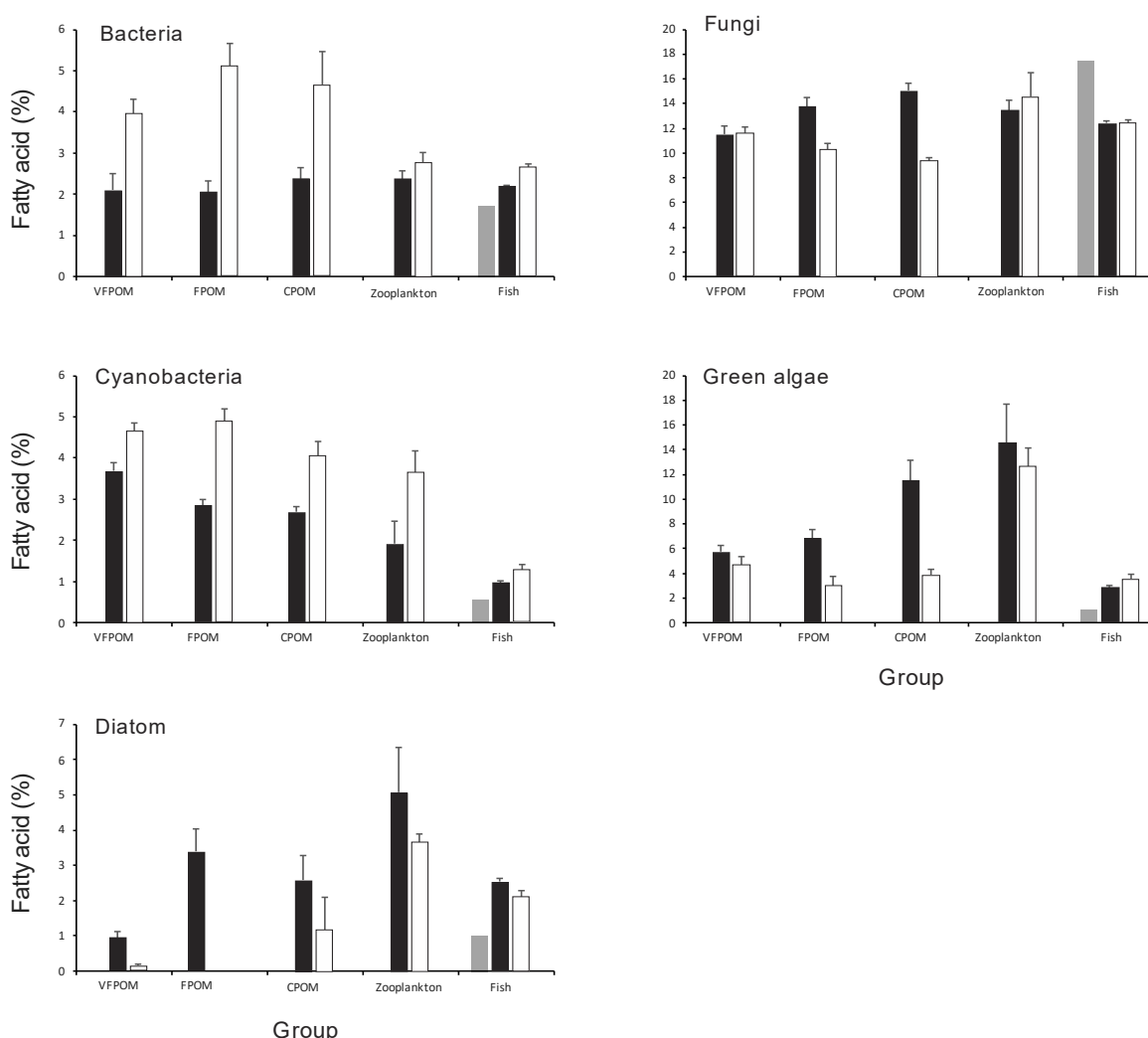

Figure 7. Mean percentage (± 1 S.E.) of fatty acid biomarkers in different substratum sizes and animal groups from Bunnor mesocosms (black columns) and Old Dromana mesocosms (white columns). Grey columns indicate the fatty acid biomarkers present in Murray cod larvae at the start of the experiment.

Discussion

We sought to identify the basal food sources for Murray cod in mesocosms with soils from wetlands with differing long-term hydrologic regimes. We hypothesised that on flooding, the emergence of invertebrates from wetland sediments and subsequent assemblages and their food value would differ between wetlands. Hence, the diet, chemical composition, growth and survival of Murray cod larvae would differ between wetlands. We used multiple lines of evidence including C:N ratios, FA profiling and PUFA concentrations to identify and characterise the nutritional value of food sources.

Our first hypothesis that the invertebrate assemblages would differ between the two wetland sediments was supported. The different fauna associated with different wetlands is reinforced by

many other studies which suggest differences in hydrology and spatial habitat heterogeneity influence invertebrate abundances and diversity e.g. (Frisch et al. 2005, James et al. 2008, Lindholm et al. 2009). The main differences in the taxa between the wetlands were more Chironomidae in the OD wetland mesocosms and more Daphnidae, Ostracoda and Macrothricidae in the Bunnor wetland mesocosms. Diets of Chironomidae differ between genera but fine particulate organic matter and benthic algae are the main feeding resource of detritivores (Butakka et al. 2016, Ruiz et al. 2018). *Daphnia* feed on green algae, bacteria and cyanobacteria (Gophen and Geller 1984, Thys et al. 2003, Oberholster et al. 2006). Diets of Macrothricidae and Ostracoda remain undocumented but as they occupy as similar habitat as *Daphnia* their food sources should be comparable, at least in our experimental mesocosms. Given the differences in invertebrate assemblages and their differing diets it was plausible that their food value to higher order consumers would differ as well. Our second hypothesis that food value of invertebrates to higher consumers would differ between wetlands was partially supported as the percentage of PUFAs was significantly greater in the Bunnor mesocosms, however, the C:N ratios were not significantly different between the mesocosms.

Our third hypothesis that survival of Murray cod larvae would be greater in the wetland with prey of greater nutritional value (based on PUFAs) was supported, however, prey density was also greater in the Bunnor mesocosms. It is therefore unclear if the greater survival is explained by density of prey rather than quality. It is unknown if the nutritional value of the benthic invertebrates differed between wetlands as we did not collect enough tissue material for analyses. However, there was no significant relationship between the density of the benthos and Murray cod survival. The minimum densities of both benthic and planktonic invertebrates required to support Murray cod larval survival are unknown but (King 2004) suggested 250 to 500 individuals L^{-1} were required to support growth. In our experiment survival declined below 50 animals L^{-1} particularly in the OD mesocosms.

Murray cod larvae appeared to be selective in the prey they ate based on the Manly-Chesson index α for the different taxa. Of the prey groups planktonic Chironomidae and benthic Chydoridae appeared to be preferred in both wetland mesocosms. In contrast, Macrothricidae/Daphniidae in the OD mesocosms and all Ostracoda seemed to be avoided, although they appear in the diets of both natural and aquacultured Murray cod larvae (Ingram and De Silva 2007, Kaminskas and Humphries 2009). The feeding preferences of the cod larvae may explain the change in the benthic invertebrate assemblage structure in the mesocosms during the experiment. (Kaminskas and Humphries 2009) noted that the natural diet of Murray cod larvae is dominated by benthic invertebrates. However, they also state that the majority of the dietary studies of the larvae of Murray–Darling native fish under aquaculture conditions have reported a diet largely based on pelagic prey items. It is unclear why the larvae were selectively feeding in our mesocosms. It may be that the different taxa have different nutritional values but although the invertebrate assemblage structure differed between wetlands their nutritional value (in terms of their C:N ratio and percentage of PUFAs) was the same. Prey selectivity has been previously observed in Murray cod larvae and other juvenile percichthyid species (Ingram and De Silva 2007). The reason for prey selectivity remains unclear, although as larvae get larger, they can tackle larger prey items. However (Ingram and De Silva 2007) found that although bigger fish had access to a larger variety of prey they still consumed smaller prey and suggested that fish may learn to utilise familiar prey size classes even when larger prey are present (Hansen and Wahl 1981).

Based on the FA indicators of different potential basal food sources, the green algae biomarker showed the greatest increase in the Murray cod from the start to the end of the experiment, suggesting that it is the main basal food source. However, the cyanobacterial and diatom indicators also increased suggesting that a combination of basal food sources are important for the cod. Fatty acid analyses showed that there was no significant differences in any of the biomarkers between wetlands suggesting the basal food sources are the same. The importance of green algae to the mesocosm food webs is supported by the well documented literature suggesting algal food sources

are of high quality because of their high content of polyunsaturated fatty acids e.g. (Volkman et al. 1989, Taipale et al. 2012, Freese and Martin-Creuzburg 2013, Guo et al. 2016).

Our results suggest that flooding of wetlands can support enough zooplankton to sustain native fish species. Mesocosm experiments provide an important bridge between smaller, more tightly controlled, microcosm experiments (which can suffer from limited realism) and the greater biological complexity of natural systems (in which mechanistic relationships often cannot be identified) (Stewart et al. 2013). The relationship between Murray cod larval survival and zooplankton densities suggests that the timing of flooding of a wetland is important so the wetland can produce enough food for fish. The Bunnor and OD wetlands appear to produce invertebrates of sufficient nutritional value to provide adequate food for larval cod growth and that the most important basal food source is green algae. However, it is unclear if our results can be extended to other wetland systems and we encourage further research both into the relationship between length of wetland flooding and invertebrate densities in other systems. We also encourage research into the mechanistic pathways in which green algae carbon is transferred through food webs to higher order consumers in wetland systems to help generalise our results to other wetlands and support the management of wetlands through the timing and duration of flooding from environmental water.

Acknowledgements

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Appendix 1: Research Activity Reports

Appendix 1.3 Basal resource transfer efficiency between a range of basal resources and to first-order consumers (mesocosm experiments)

1.3.2 The use of fatty acids in freshwater ecological research

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Abstract

Fatty acids (FA) are a class of lipids that act in essential functions in trophic interactions in food webs and for physiological processes in all organisms. The literature regarding the roles of fatty acids has a long history in marine systems. Here, we present a review of the scientific literature on the use of FA in freshwater ecology limited to experimental studies. We identified 177 papers that have used FA to either examine food webs, anthropogenic disturbance, subsides between ecosystems, seasonal or spatial variation and as biomarkers. The majority of research has concerned the use of FA for examining food web structures and has been subject to several recent reviews so is not covered here. We examined the remaining research topics and identified six areas where FA could be used to develop new knowledge in freshwater ecology. Our review assists natural resource management by highlighting consistencies among studies but also identifies where research is inconclusive and requires further work to establish ecological generalisations.

Introduction

Fatty acids (FA) are a class of lipids consisting of a long hydrocarbon chain attached to a carboxyl group (a carbonyl group (C=O) that has a hydroxyl group (O-H) attached to the carbon atom) (Ratnayake and Galli 2009). They act in essential functions in trophic interactions in food webs and for physiological processes in all organisms, including antecedents to anti-inflammatory eicosanoids (Pernet et al. 2007), preservation of cell membranes and their functions (Guschina and Harwood 2009), and for metabolism including energy storage (Dalsgaard et al. 2003). The number and composition of the FA (or FA profile) in any particular material or organism is influenced by many factors, including but not limited to, environmental influences (e.g. temperature), phylogeny, diet and metabolism e.g. (Piorreck et al. 1984, Burns et al. 2011, Lang et al. 2011).

Two forms of fatty acids, omega-3 (termed n-3) and omega-6 (n-6) poly-unsaturated fatty acids (PUFA), are produced almost exclusively by phytoplankton, macrophytes, and plants (Dalsgaard et al. 2003), and are critical types of nutrients for heterotrophs, including invertebrates, fish, and humans (Arts et al. 2001). Because heterotrophs cannot make n-3 and n-6 PUFA (Parrish 2009), these molecules are thought to be 'essential' fatty acids (EFA) for consumers. Aquatic (marine and freshwater) ecosystems are the primary source of n-3 fatty acids in the environment, therefore supporting both aquatic and terrestrial heterotrophs via the trophic transfer of these key EFA through food webs (Gladyshev et al. 2013). Fatty acids are a promising biochemical constituent to use as a proxy for ecosystem-scale food quality because there is wide ranging research detailing that growth and reproduction of many aquatic consumers may be constrained by certain EFA (Galloway and Winder 2015).

The literature regarding the roles of fatty acids has a long history in marine systems (Dalsgaard et al. 2003, Suzumura 2005, Mrozik et al. 2014). Here, we present a review of the scientific literature on the use of FA in freshwater ecology limited to experimental studies.

Methods and Results

I retrieved relevant publications by conducting a search of two scientific databases, Web of Science® and Scopus®, using the search phrase ("fatty acid" AND freshwater). The searches yielded 1120 and 1645

citations respectively. A large number of references were mainly concerned with human nutrition, aquaculture and/or biofuels. Narrowing these references to work carried out in freshwater ecosystems yielded 177 references (Table S1). Papers just describing the fatty acid composition of material or biota were ignored e.g. (Bell et al. 1994). The earliest paper was published in 1979 (Figure 1). This paper concerned the effects of diets with different dietary fatty acids and temperature on the production of a cladoceran (D'Abramo 1979). However, the majority of papers have been published since 2009. The increase in publications is probably due to technological advances in the ability to analyse FA profiles in biological material (Tang and Row 2013) but also the realisation by the scientific community of the value of FAs in environmental research.
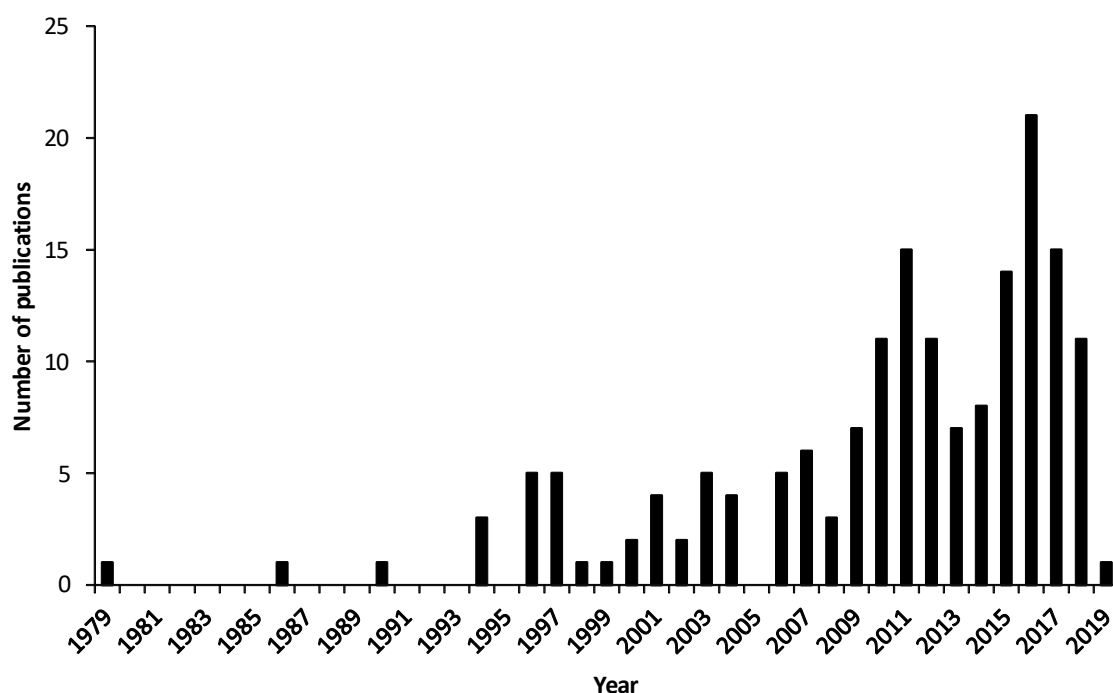

Figure 1. Number of publications by year using fatty acids in freshwater ecological research.

The majority of publications have used laboratory and mesocosm experiments or taken place in lentic habitats such as ponds and lakes (Table 1). Research has taken place in a large number of subject groups, with the majority of work being conducted on zooplankton and phytoplankton. In contrast, we only identified one (0.6%) paper for each of amphibians, birds and mammals. Most research has taken place with only one or two subject groups, with only eight papers concerning four or more biotic groups. Each of these papers were mainly concerned food webs and the different groups were examined for trophic linkages between them e.g. (Czesny et al. 2011, Sanseverino et al. 2012, Volk and Kiffney 2012, Carassou et al. 2017). Fatty acids have been used for a range of topics, but over half of the research has concerned food webs and the links between trophic groups. Reviews for food webs have recently taken place for both lentic and lotic habitats e.g. (Guo et al. 2016, Brett et al. 2017), so we do not review this material further. My review concentrates on the use of FA in other subject areas and highlights potential future work.

Table 1. Percentage of publications in different habitats, subject groups, number of groups and research topics.

Habitat	Publications (%)	Subject Group	Publications (%)	Number of subject groups	Publications (%)	Major themes	Publications (%)
Laboratory/mesocosm	37.1	Sediment	5.3	1	12.4	Food web	53.5
Lentic	41.2	Seston	12.9	2	27.6	Disturbance	28.2
Lotic	24.7	Leafpacks	2.9	3	8.2	Subsidy	4.7
Estuary	1.8	Periphyton	10.0	4	4.1	Seasonal or spatial variation	8.8
		Cyanobacteria	4.1	5	0.6	Biomarkers	11.2
		Bacteria	8.8	6	0.6		
		Phytoplankton	25.9				
		Aquatic plant	0.6				
		Zooplankton	41.8				
		Benthic invertebrates	16.5				
		Mollusc	11.8				
		Amphibians	0.6				
		Fish	22.9				
		Birds	0.6				
		Mammal	0.6				

Effects of disturbance or environmental factors on fatty acid composition

Climate change and temperature

The main effect of climate change or global warming on lentic ecosystems is thought to be increasing temperatures as climate modelling suggests that both surface air temperatures and the frequency of extreme heat days will continue to increase (Team et al. 2014). This effect is likely to be most pronounced in water bodies with large surface-to-volume ratios (i.e., small ponds and large shallow lakes (McKee et al. 2002, Van Doorslaer et al. 2007). (Hixson and Arts 2016) conducted an exploratory data synthesis with 952 fatty acid (FA) profiles from six major groups of marine and freshwater phytoplankton. They found temperature was strongly correlated with a decrease in the proportion of n-3 long-chain polyunsaturated FA (LC-PUFA) and an increase in omega-6 FA and saturated FA. (Hixson and Arts 2016) thus suggested that a reduced production of these EFA, is predicted to negatively affect species that depend on these compounds for optimum physiological function and that would lead to cascading effects throughout the world's ecosystems. However, this work was a correlative study and there may have been other confounding effects causing their findings. These include, but are not limited to, their literature search may have imposed a bias on the types of phytoplankton that were included and variation within groups is expected given that the data came from studies that used a variety of different culture methods and had a range of different study objectives.

Actual experimental evidence on the effects of temperature, and therefore presumably climate change, on the FA composition in freshwater ecosystems and biota is limited and contradictory. Temperature is assumed to have strong influence on the quantity and quality of FA in both marine and freshwater phytoplankton as acclimatisation to increasing temperature involves decreasing PUFA membrane content, while, simultaneously, increasing saturated fatty acids (SFA), to maintain cell membrane structural rigidity (Rousch et al. 2003, Fuschino et al. 2011). Several studies have shown that PUFA increases in green algal phytoplankton from low temperatures (~15°C) to moderate temperatures (~20°C) but then declines with greater temperatures (~25°C) (Renaud et al. 2002, Hodaifa et al. 2010, Fuschino et al. 2011, Sikora et al. 2014, von Elert and Fink 2018). However, temperature effects on fatty composition may differ with freshwater phytoplankton species as it does for marine species (Thompson et al. 1992).

The effects of temperature on phytoplankton being passed onto higher order consumers will depend greatly on whether the relevant traits of both producers and consumers will be affected asymmetrically or not (Dell et al. 2014) and may be regarded as the result of an interaction of direct physiological effects of temperature and indirect effects due to changing interplay between populations (Zhang et al. 2017). We identified two studies in our literature search that have examined the effects of changed FA composition of phytoplankton derived from temperature changes on higher order consumers and had opposing findings. The first study hypothesized that a shift to cyanobacterial communities caused by climate change would reduce the efficiency of feeding and growth of zooplankton (Przytulska et al. 2015). They tested the growth and FA composition of Daphnia from regions with differing temperature and fed either cyanobacteria or green algae. They found that there was a significant negative effect of increased temperature and cyanobacterial food on zooplankton fatty acid content and composition. The combined effect of temperature and food quality on the performance of the low temperature Daphnia was greater than their effects added separately, further indicating the potentially strong indirect effects of climate warming on aquatic food web processes.

The second study examined the relative influences of abiotic selection and food web effects associated with climate change on intraspecific differences and interrelationships between HUFA content, body size, and fecundity of freshwater copepods (Charette and Derry 2016). In artificial ponds climate-driven temperature changes favoured the coexistence of a diversity of phytoplankton species with different temperature optima and nutritive quality. The change resulted in positive relationships between temperature, copepod DHA content and body size. Temperature correlated positively with algal biovolume, and arbitrated relationships between copepod HUFA content, body size and fecundity. The presence of brook trout further increased these positive effects in warm ponds, likely through nutrient input and stimulation of phytoplankton resources. These results suggest that climate change may have previously unrecognised positive effects on higher order consumers.

Altered land use and agriculture

Altered land use and agricultural activities have the potential to significantly alter the physical, chemical, and biological properties that comprise stable and healthy stream ecosystems. Their effects include riparian vegetation removal or alteration, elevated sediment loads, dissolved nutrients, and inputs of environmental pollutants (Ehrman and Lamberti 1992, Osborne and Kovacic 1993, Strayer et al. 2003). Alteration of the stream environment has the potential to alter the basal food sources and therefore the nutritional quality and FA profiles of primary producers and higher order consumers. We identified six papers that examined the potential of land use change and agriculture to affect the FA composition in stream organic matter and biota.

Two studies specifically examined agricultural land use effects on primary producers and have similar findings. (Boëchat et al. 2011) examined how the fatty acids benthic microbial mats differed between tropical streams located in protected pristine and agricultural savannah areas. Agricultural streams had lower total biomass of benthic microbial mats than pristine streams. However, the higher concentrations of essential polyunsaturated fatty acids, such as linoleic acid (LIN, 18:2ω6), α-linolenic acid (ALA, 18:3ω3), and eicosapentaenoic acid (EPA, 20:5ω3), that were observed in agricultural streams suggest enhanced lipid complexity and a higher nutritional quality of the microbial community relative to pristine streams. (Whorley and Wehr 2018) investigated the nutritional contribution of benthic algae in the form of fatty acids (FAs) in benthic algae in streams with different ages of improved environmental management of practices (EMP) versus pristine streams. Their results indicated that EMPs were able to reduce agricultural effects, as evidenced by a significantly lower benthic algal total FA content in reference than in agricultural streams. However, FA content did not differ between EMP-age categories, a result suggesting that EMPs were effective within one to two years.

The four remaining studies examined the effects of FA composition of suspended organic matter in streams and rivers. (Boëchat et al. 2014) analysed land-use effects on the FA composition and concentrations in suspended particulate organic matter (SPOM) along a fourth-order tropical river. Urbanisation was the only the land-use category correlating with both FA composition and concentrations. Higher concentrations of saturated FAs, especially C16:0 and C18:0, which are the main components of domestic sewage, were observed at sampling stations downstream of urban centres. They suggest that FA can be used as biomarkers to identify anthropogenic disturbances. (Larson et al. 2013) investigated variation in the fatty acid (FA) composition of seston and primary consumers within and among 11 tributary systems of Lake Michigan, USA. They hypothesised that among-system and among-habitat variation in FAs at the base of food webs would be related to algal production, which in turn is influenced by three different land uses. Of the three land cover characteristics, only intense land use appeared to be strongly related to seston and consumer FA and this effect was only strong in river mouth and nearshore lake sites. River seston and consumer FA composition was highly variable, but that variation does not appear to be driven by the watershed land cover characteristics. (Lu et al. 2014) compared the FA composition of POM from eight headwater streams influenced by different types of watershed land use. The contribution of bacterial fatty acids differed significantly among sampling times, but not among land use types. However, the concentrations of total long-chain polyunsaturated fatty acids, eicosapentaenoic acid and arachidonic acid showed that the nutritional value of POM in forest streams was lower than in urban streams. (Ngugi et al. 2017) used lipid biomarkers in the suspended particulate OM (SPOM) at the seven river discharge sites in Lake Victoria, in an effort to evaluate the potential of upstream catchment land use. At sites receiving discharge from industrial effluent the FAs were dominated by short chain FAs (SCFAs), polyunsaturated FAs (PUFAs), monounsaturated FA (MUFAs) and branched FAs. Sites receiving discharge from substantial agricultural activity in the catchment were dominated by long chain FAs (LCFAs) reflecting vascular plant terrigenous input. They suggested that human land use in upstream watersheds affect the source, composition and distribution of SPOM discharged by rivers but may also change the quality of SPOM. Of the six studies we identified in this section only (Larson et al. 2013) investigated whether changes in the basal food source was transferred to consumers. Future research is required to evaluate if the effects of altered land use and agriculture influences the production of species higher in the food web.

Herbicides

We identified three studies that examined the effects of herbicides on the fatty acid composition and all involved green algae. (Chalifour et al. 2014) investigated the effect of low temperature on the toxicity of Norflurazon (Nf) and fluridone (Fd) herbicides on a *Chlamydomonas reinhardtii*. These two herbicides are widely used for the control of grasses and invasive aquatic weeds. The fatty acid composition was modified by temperature and the level of unsaturation noticeably increased at 15 compared with 25ºC. At 8ºC, however, despite a 2.4 times decrease in fatty acid content, the unsaturation level was similar to 25ºC acclimated cells. Monounsaturated fatty acids increased concomitant with a decrease in polyunsaturated fatty acid in the Nf treatment at 25ºC suggesting a decrease in the nutritional value of the algae. (El-Sheekh et al. 1994) showed the herbicide atrazine concentrations induced a general inhibition on growth, photosynthesis, and dark respiration in the green alga Chlorella kessleri. The herbicide had preferential effect on the associated fatty acid composition of total and phospholipids. After 24 and 48 hr the herbicide stimulated fatty acids synthesis at concentrations where photosynthesis was inhibited. Meanwhile, stearic and miristic acids disappeared at 15 µM after 24 hr and the total polyunsaturated fatty acids were not affected after 48 hr. Fatty acid synthesis was sensitive to treatment at 72 hr by 5 and 10 µM atrazine, whereas the total saturated fatty acids were completely inhibited. Both studies therefore demonstrated a change in the FA composition of algae with herbicide exposure. The third study examined whether consumers of algae could be affected by herbicide application.

(da Silva et al. 2016) compared the reproductive potential of *Daphnia* fed a diet of *Raphidocelis subcapitata* exposed to the herbicide pendimethalin with control mesocosms. The chemical composition of *R. subcapitata* exposed to the herbicide revealed a slight decrease on total fatty acid levels, with a particular decrease of essential omega 9 monounsaturated fatty acids. Female D. magna exposed to the herbicide diet experienced a 16% reduction in reproduction, measured as the total number of offspring produced per female. Although they suggest it is difficult to discriminate the contribution of the pesticide (as a toxic agent transferred through the food web) from that of the food with a poor quality-compromised by the same pesticide, there are no doubts that, under environmentally relevant concentrations of pesticides, both pathways may compromise the populations of freshwater grazers in the long term, with consequences in the control of the primary productivity of these systems.

Nutrients and cyanobacterial blooms

The effects of both increased and decreased nutrient loading on FA in biota in lentic and lotic systems have been examined in the literature. Changes in nutrient inputs can have overwhelming effects upon the quality of receiving waters (Carpenter et al. 1998, Correll 1998). The most common effect of increased important nutrients, nitrogen and phosphorus, to aquatic ecosystems is an increase in the abundance of algae and aquatic plants and a shift in phytoplankton composition to bloom-forming species, many of which may be toxic, or which may not be consumed effectively by aquatic grazers (Smith 2003).

We identified six studies that examined increased nutrient effects in lentic environments, two of these only examined the direct effects on algal species. (Hu and Gao 2006) tested the effects of different levels of nitrogen (N), phosphate (P), salinity and temperature on the green algae *Nannochloropsis* sp. Increased levels of N and P raised protein and polyunsaturated fatty acids (PUFAs) contents but decreased carbohydrate, total lipid and total fatty acids (TFA) contents. (Piepho et al. 2012) tested the influence of phosphorus (P) supply, light intensity and temperature on fatty acid (FA) concentrations of four freshwater algae. The effect of increased P on FA was complex and was affected by both light intensity and temperature and differed between species. For example, changes in several saturated and unsaturated FA concentrations with light were more distinct in the low-P treatments than in the high-P treatments. Interactive effects of temperature and P supply on various FA concentrations were observed in all four species, but there was no consistent pattern. However, the TFA, SAFA and MUFA content of three of the algal species decreased with P addition.

Three studies examined the effects of eutrophication changes in FA in basal food sources on higher order consumers. (Larson et al. 2016) examined the effects of eutrophication in Lake Erie on the border of the USA and Canada on secondary production and FA content in freshwater mussels. They found secondary production was highest in the more eutrophic sites and lowest in the less eutrophic sites. Mussel tissues in

the eutrophic sites also included more eicosapentaenoic and docosapentaenoic fatty acids (EPA and DPA, respectively), but fewer bacterial FAs, suggesting more algae at the base of the food web in the more eutrophic sites. Cyanobacterial abundance among sites was not correlated to secondary production but was positively related to EPA and DPA content in the mussels, suggesting more of these important FAs in locations with more cyanobacteria. These results suggest that growth of secondary consumers and the availability of important fatty acids in the more eutrophic waters. (Müller-Navarra et al. 2000) showed that low transfer efficiencies between primary producers and consumers exist during cyanobacteria bloom conditions caused by eutrophic conditions are related to low relative eicosapentaenoic acid ($20:5\omega3$) content of the primary producer community. Zooplankton growth and egg production were strongly related to the primary producer $20:5\omega3$ to carbon ratio. This indicates that limitation of zooplankton production by this essential fatty acid is of central importance at the pelagic producer–consumer interface. Similarly, (Bartsch et al. 2017) demonstrated that increased nutrient and sediment loading in rivers have caused observable changes in algal community composition, and thereby, altered the quality and quantity of food resources available to native freshwater mussels. They suggested areas dominated by cyanobacteria caused by eutrophication may not provide sufficient food quality to promote or sustain mussel growth.

One study examined sedimentary organic matter composition in lakes with different trophic states (as indicated by N and P concentrations) and attempted to identify the sources using fatty acid biomarkers (Xu et al. 2014). Organic matter incorporated into sediments of freshwater lakes is generally derived from a variety of sources. The fatty acid profile data of examined freshwater lakes indicate that there were several organic matter sources including microalgae, aquatic plant, terrestrial plant and bacteria. They found terrestrial plant derived organic carbon was a useful predictor for sediment TOC. However, with increasing trophic state the aquatic source contribution to sedimentary organic matter pool increased.

We found two studies that examined the effects of decreased nutrients on FA in lentic systems. (Ahlgren et al. 1998) in laboratory experiments demonstrated that phosphorus limitation affects both quality and PUFA in diatoms and green algae. Phytoplankton grown at low P concentrations had reduced PUFA but increased SAFA. (Hartwich et al. 2012) compared the elemental and biochemical composition of natural seston in oligotrophic and mesotrophic phases of a lake. They also conducted *Daphnia* growth experiments using the oligotrophic seston supplemented with omega 3 PUFA. In the oligotrophic phase, particulate carbon and phosphorus concentrations were lower, fatty acid concentrations were higher, and the taxonomic composition of phytoplankton was less diverse. The limitation by food quantity (in terms of carbon) and quality (i.e. α-linolenic acid) during the mesotrophic phase changed to a complex multiple nutrient limitation mediated by food quantity, phosphorus, and omega-3 fatty acids in the following oligotrophic phase. Growth of *Daphnia* was limited by either phosphorus or omega-3 fatty acids. They suggested that seasonal and annual changes in nutrient availabilities can create complex co-limitation scenarios consumers have to cope with, which consequently may also affect the efficiency of energy transfer in food webs.

We identified two studies that examined eutrophication in lotic ecosystems. (Ziegler and Lyon 2010) used in situ 13C-labeling experiments conducted in six streams representing a gradient in nutrient enrichment to explore how FA, stoichiometry, and the composition of active biofilm phototrophs may regulate C cycling in epilithic biofilms. Carbon cycling was tracked through epilithic biofilm communities by assessing net primary production (NPP) and 13C-labeling of biofilm phospholipids fatty acids (PLFA). The absolute quantities of PUFA was highest in the two most nutrient rich sites. In addition, the quantity of new C incorporated into PUFA was generally higher in the two more nutrient-rich streams. (Dalu et al. 2016) compared essential fatty acids (EFAs) in the phytobenthos (benthic algae) growing on different substrate types (bricks, clay tiles, rocks, macrophytes, and sediments) at 2 river sites subject to differing using nutrient concentration. They hypothesized that the fatty acid (FA) content of phytobenthos changes in response to shifts in local nutrient availability but not substrate type. EFA content ($18:2\omega6$, $18:3\omega3$, $20:4\omega6$, $20:5\omega3$, and $22:6\omega3$) in the phytobenthos differed overall among substrates, sites, and seasons and was generally greater in summer than in autumn and winter. EFA content was significantly greater on artificial than natural substrates and was greater at the nutrient-enriched downstream site than at the upstream site. These studies suggest that eutrophication in streams and rivers increases the nutritional

value of basal food sources. However, future research is required to examine how the changes would relate to the production of higher order consumers.

We found three investigations that examined the FA consequences of cyanobacterial blooms that did not necessarily consider eutrophication per se. (Von Elert et al. 2003) in controlled growth experiments, showed that the low C transfer efficiency of coccal and filamentous cyanobacteria to the keystone herbivore *Daphnia* is caused by the low sterol content in cyanobacteria, which constrains cholesterol synthesis and thereby growth and reproduction of the *Daphnia*. Estimations of sterol requirement in Daphnia suggest that, when cyanobacteria comprise more than 80% of the grazed phytoplankton, growth of the herbivore may be limited by sterols and Daphnia may subsequently fail to control phytoplankton biomass. Dietary sterols therefore may play a key role in freshwater food webs and in the control of water quality in lakes dominated by cyanobacteria. (Wacker and Martin-Creuzburg 2007) suggested that cyanobacteria feature a deficiency in some essential lipids that leads to a decline in the growth and fecundity of *Daphnia*. Thus, they investigated *Daphnia magna*'s allocation of lipids such as polyunsaturated fatty acids (PUFAs) and cholesterol during an experimental non-toxic cyanobacterial bloom. They found considerable maternal investment of the particularly important omega-3 (n-3) PUFAs, especially eicosapentaenoic acid (EPA), into the eggs. The concentration of EPA was 2.4-fold higher in eggs than in somatic tissue, a cumulative effect, which was not significantly changed under cyanobacterial food conditions. Under deprived food conditions, *D. magna* not only decreased the number of eggs produced but, in principle, reduced the previously high concentrations of EPA in both eggs and somatic tissues to a similar degree. In contrast to EPA, the concentrations of α-linolenic acid and cholesterol, although lower than EPA, were more homeostatic in eggs than in somatic tissues, in which concentrations decreased. When food quality was enhanced, *D. magna* were able to recover completely the fatty acid concentrations in their somatic tissues and eggs. They demonstration PUFAs are primarily needed for reproduction.

(Groendahl and Fink 2017) conducted a laboratory experiment in which they fed the herbivorous freshwater gastropod *Lymnaea stagnalis* single non-toxic cyanobacterial and unialgal diets or a mixed diet to test if diet-mixing may enable these herbivores to control non-toxic cyanobacterial mass abundances. The treatments in which *L. stagnalis* were fed non-toxic cyanobacteria and a mixed diet provided a significantly higher shell and soft-body growth rate than the average of all single algal. However, the increase in growth provided by the non-toxic cyanobacteria diets could not be related to typical determinants of dietary quality such as toxicity, nutrient stoichiometry or essential fatty acid content. The authors suggest that their results strongly contradict previous research which describes non-toxic cyanobacteria as a low quality food resource for freshwater herbivores in general and that that freshwater gastropods may be able to control benthic non-toxic cyanobacteria blooms.

Invasive species

We identified eight papers that used FA to define the roles of invasive species on freshwater ecosystems. Four of these involved invasive bivalve gastropods, two fish and two crustaceans. The first bivalve study assessed the capability of the invasive freshwater bivalve *Corbicula fluminea* to process and modify the quality of pelagic food sources for subsequent use by benthic invertebrates (Basen et al. 2013). They ran a growth experiment in which juvenile *Gammarus roeselii* were raised either directly on pelagic algae or on the same algae biodeposited by the bivalve either as faeces or pseudofaeces. They showed that growth and survival of *G. roeselii* significantly improved when algae was offered as biodeposited material and suggested that this clam-mediated upgrading of food quality is due to both an increased bioavailability of pelagic food particles, which are packed in mucus during clam processing, and an increased dietary provisioning with essential lipids (sterols and polyunsaturated fatty acids) originating from the clams. The second study involving a bivalve investigated *C. fluminea* nutritional requirements for essential lipids in a standardized growth experiment (Basen et al. 2011). Juvenile clams were fed one of three cyanobacterial species or one of two green algae. Somatic growth rates were then correlated with elemental (C:N and C:P) and biochemical (sterol and fatty acid content) components of the food sources and clam tissue. Somatic growth rates were significantly higher when juveniles were fed green algae than when fed cyanobacteria. Linear regression analyses revealed significant positive relationships between somatic growth rates and dietary sterol and polyunsaturated fatty acid content. The result suggests that the growth of *C. fluminea* is partially dependent on the availability of these essential lipids in the diet. The third study compared the

feeding spectra of the an invasive bivalve (*Dreissena polymorpha*) with a coexisting native bivalve (*Unio tumidus*) using a fatty acid (FA) marker analysis (Makhutova et al. 2013). Analyses of FA trophic markers in bivalve tissues showed that *U. tumidus* and *D. polymorpha* mainly consumed algae (greens, diatoms, and dinoflagellates), cyanobacteria, and detritus particles enriched with bacteria. According to the multivariate statistical analysis, the two species had different feeding ranges: *D. polymorpha* fed on planktonic sources, while *U. tumidus* mostly consumed food sources of benthic origin, mainly detritus. In addition, U. tumidus and Dreissena species differed in percentages of long-chain polyunsaturated FAs of n-3 and n-6 families and specific FAs which they could synthesize (20:1n-13 and 22:3 Δ7,13,16). They concluded that the different feeding spectra of the two species and the ability of *U. tumidus* to the synthesis of specific FAs, might be the basis for the successful coexistence of native species and invaders. The fourth study assessed the effects of the invasive bivalve *Dreissena polymorpha* on the physiology of the native bivalve *Lampsilis siliquoidea* using FA (McGoldrick et al. 2009). Concentrations of total lipid and of individual fatty acids in *L. siliquoidea* and therefore physiological condition were not significantly correlated with *D. polymorpha* infestation, but fatty acids do provide a possible explanation for differences in glycogen concentrations at different sites in the Great Lakes. Unionids from sites in US waters had higher levels of palmitoleic acid (16:1n-7) and eicosapentaenoic acid (20:5n-3) in their tissues, suggesting that they consume a diet rich in diatoms. Unionids collected from on the Canadian side of the delta had higher concentrations of α-linolenic acid (18:3n-3) and linoeic acid (18:2n-6), which is indicative of a diet rich in green algae and cyanobacteria, respectively. These results suggest that diatoms are more abundant in less productive US waters, which receive most of the water flowing out of Lake Huron, whereas green algae and cyanobacteria make up larger portions of the planktonic community in the more eutrophic Canadian waters.

The first study to use FA in an invasive fish species examined the effect of dissolved organic carbon (DOC) concentrations of lake water (Scharnweber et al. 2016). They conducted a field study to investigate the connection between morphological divergence and polyunsaturated fatty acid (PUFA) composition of *Perca fluviatilis* from six lakes across a gradient of DOC concentration. They expected a decrease in the concentration of fish PUFAs due to the restructuring effects of DOC on aquatic food webs. Proportions of specific PUFAs (22:6n-3, 18:3n-3, 20:5n-3, and 20:4n-6) did not differ in perch caught in clear and brown-water lakes, indicating no severe limitation of essential fatty acids for perch inhabiting brown water lakes. The second fish study examined the potential effects of the alien bigheaded carp on fatty acid profiles of multiple trophic levels in large rivers (Fritts et al. 2018). Fatty acid concentrations in seston were positively associated with omega-3 highly unsaturated fatty acids, indicating that these locations had abundant, high-quality basal food resources despite hosting the greatest bigheaded carp densities. Fatty acid profiles of freshwater mussels were similar to the fatty acid values in the seston and were not influenced by bigheaded carp abundances. Hydropsychid caddisflies and bluegill fish did not differ significantly in total fatty acids or percent lipid among spatial locations, indicating that omnivorous species may be relatively unaffected by bigheaded carps. Gizzard shad (a zooplankton predator), however, exhibited the lowest fatty acid concentrations in the locations with the highest relative bigheaded carp densities. Zooplankton densities are negatively affected bigheaded carps' establishment, which may explain the disconnect between the gizzard shad fatty acids and the plentiful, high-quality phytoplankton those rivers.

The first of two papers describing the effects of an invasive crustacean using FA examined the effects of *Bythotrephes longimanus* on a resident crustacean species (*Mysis relicta*) (Nordin et al. 2008). Significant differences in FA amount and composition of *M. relicta* were found between invaded and non-invaded lakes, and among lakes within these groups. Generally, *M. relicta* in non-invaded lakes had higher concentrations of C16:0, C18:1n9c, C18:2n6c (linoleic acid), C18:3n3 (α-linolenic acid) and C20:4n6, while *M. relicta* in invaded lakes had higher concentrations of C22:6n3. However, differences in FA profiles and gut contents of *M. relicta* between invaded and non-invaded lakes are consistent with competition for Cladocera in the presence of the invader rather than pre-existing differences among lakes. They conclude that the diet of *M. relicta* is affected by the invasion of *B. longimanus.* The second paper examined the FA profile of the invasive Ponto-Caspian mysid *Limnomysis benedeni* (Fink 2013). The author fed this species green algae and young *Daphnia* in mesocosms and found the mysid can biosynthesise eicosanoid FA because they were not present in the experimental foods. (Fink 2013) suggested that invasion of mysids

capable of trophic upgrading of dietary fatty acids might have positive effects on the fitness of planktivorous fish.

Material and energy subsidy between ecosystems

Fatty acids have been used to elucidate the connection and subsidies at freshwater and terrestrial and freshwater and marine interfaces. Our literature search identified eight studies using FA for examining the subsidies of material and energy between ecosystems. We do not include the literature that has been already been reviewed examining the importance of terrestrial litter inputs to freshwater food webs.

(Heintz et al. 2004) suggested that adult salmon returning to their natal streams represent a significant source of energy, nutrients, and biochemicals. They tested this hypothesis by adding different levels of dead pink salmon Oncorhynchus gorbuscha to stream mesocosms and testing the for the fatty acid composition of resident reared Coho salmon O. kisutch. The lipid content and triacylglycerols of the Coho salmon increased with increasing carcass density whereas phospholipids decreased. Increased amounts of triacylglycerols accounted for most of the lipid increase. In addition to increasing in concentration, the fatty acid composition of the triacylglycerols also changed with carcass density. Triacylglycerols of juvenile Coho salmon from the control streams had significantly higher omega-3: omega-6 ratios as a result of fivefold and sixfold increases in the concentrations of eicosapentanoic and docosahexanoic fatty acids, respectively. These data demonstrate an immediate nutritional advantage resulting from the introduction of salmon carcasses in juvenile Coho salmon rearing habitat and indicate the usefulness of fatty acid and lipid class analysis for investigating the effects of marine-derived nutrients on juvenile salmonids.

(Smits et al. 2016) investigated how sockeye salmon (Oncorhynchus nerka) spawning density and stream temperature affect the growth, body condition, and fatty acid composition of juvenile coho salmon (Oncorhynchus kisutch), a known egg predator. They compared mean body size of juvenile coho salmon and found that the largest occurred in warm streams in which sockeye salmon spawned, although overall subsidy magnitude (spawner density) had no effect on consumer body size. Individuals that consumed more salmon eggs (estimated from δ15N) were larger and had altered fatty acid composition but did not have higher relative body condition. These results indicate that effects of marine subsidies on freshwater consumers depend both on local habitat conditions and on individual variation in energy allocation.

(Samways et al. 2017) examined whether fatty acids (FAs) delivered to rivers and streams by spawning by returning Atlantic salmon (Salmo salar) are incorporated by freshwater biota. They used stream mesocosms and added marine derived nutrients from Atlantic salmon to observe FA profiles of biofilm, macroinvertebrates, and Atlantic salmon juveniles. Spawning Atlantic salmon had a positive effect on total lipid content in all trophic levels relative to controls. Fatty acid profiles of the invertebrates and salmon juveniles from the treatment stream were similar to the FA profile of the salmon eggs. They suggest that the observed responses demonstrate a change in the food web structure resulting from the establishment of this new, marine-based, basal resource. The positive effects of the marine subsidy increasing freshwater productivity are complimented by marine-based lipids that represent surplus energy and an increase in the quality of resources, thereby contributing to the diversity and health of freshwater ecosystems.

The remaining papers examined various subsidies from terrestrial sources. The first, (Masclaux et al. 2011) examined importance of allochthonous pollen deposition for zooplankton production using growth experiments on two cladocerans (Daphnia longispina and Simocephalus vetulus). The animals were fed pollen from three species (Alnus sp., Populus sp., and Cedrus sp.) and FA profiles analyses. They found that although lipid analysis revealed high polyunsaturated fatty acid (PUFA) contents in pollen, both cladoceran species exhibited suboptimal development when directly fed on pollen. The low food value of pollen was attributed to the presence of a refractory wall reducing its digestibility. In a second set of experiments, cladocerans were fed on a mixture of heterotrophic microorganisms (bacteria, chytrids, and protozoa) that had grown on pollen grains (Cedrus sp.). The introduction of microorganisms as an intermediate trophic level resulted in cladoceran growth rates that were about double those obtained on pollen alone. Hence, our findings suggest that pollen carbon could sustain zooplankton growth indirectly, and highlight the key role of microorganisms, and especially of chytrids, in transferring and upgrading pollen PUFA to higher trophic levels. The second paper builds on the first when (Masclaux et al. 2013) examined the transfer of pollen organic carbon to aquatic consumers during a pine pollen rain event on an oxbow lake. They found

microorganism concentrations were by far higher in the neuston, where pollen grains accumulated, than in the seston. Zooplankton species were also unevenly distributed in the two compartments. Bulk isotope and isotopes of fatty acid analyses showed trophic partitioning among these zooplankton species, with some of the taxa foraging specifically on neuston, where they benefit from pollen-derived carbon. Microorganisms were identified as a key element in the trophic upgrading of pollen food quality and in the transfer of pollen carbon to metazoan consumers. Pollen rains may thus contribute, as an allochthonous food pulse, to aquatic production at specific seasons, but they may also act as a structuring factor in lake habitats. The third paper, (Vargas et al. 2011) evaluated the potential sources and composition of organic matter along a lake-river-fjord corridor using stable isotope (δ13C) and lipid (fatty acid composition) biomarker analyses. A saturated FA biomarker generally ascribed to higher plants comprised one of the highest fractions of POC fatty acids along the river plumes, up to 68-86%. The authors used this result to suggest that rivers may subsidise fjord ecosystems with allochthonous contributions. The fourth study, (Taipale et al. 2016a) examined the importance of terrestrially based food sources in freshwater food webs using a combination of *Daphnia* feeding trials and FA profiles in field collected cladocerans. They showed that during phytoplankton deficiency, zooplankton (Daphnia magna) can benefit from terrestrial particulate organic matter by using terrestrial-origin carbohydrates for energy and sparing essential fatty acids and amino acids for somatic growth and reproduction. Assimilated terrestrial-origin fatty acids from shoreline reed particles exceeded available diet, indicating that Daphnia may convert a part of their dietary carbohydrates to saturated fatty acids. The proportion of low-quality diets (bacteria and terrestrial particulate organic matter) was < 28% of the assimilated carbon. The results of the two study components suggested that the incorporation of terrestrial carbon into zooplankton was not directly related to the concentration of terrestrial organic matter in experiments or lakes, but rather to the low availability of phytoplankton. The last paper, (Martin-Creuzburg et al. 2017) examined the export and therefore a terrestrial subsidy of essential polyunsaturated fatty acids (PUFA) via emerging insects from a lake. Insect emergence from varying depths and seasonal mean fatty acid concentrations in different insect groups were used to estimate PUFA fluxes. While Chironomidae contributed most to insect biomass and total PUFA export, Chaoborus flavicans contributed most to the export of EPA, ARA, and especially DHA. Applying a conceptual model developed to assess insect deposition rates on land to our insect-mediated PUFA export data revealed an average total PUFA deposition rate of 150 mg m^{-2} yr^{-1} within 100 m inland from the shore. They suggest that their data indicate that insect-mediated PUFA fluxes from lakes are substantial, implying that freshwater-derived PUFA can crucially influence food web processes in adjacent terrestrial habitats.

Seasonal or spatial variation in fatty acids

Understanding the size and extent of variation in both temporal and spatial of FA is important to identify food web functioning and changes in the nutritional value of basal food sources. Trophic organisation of complex food webs may vary at numerous spatial and temporal scales, both in terms of direct trophic connections and the underlying energy pathways that support production. The literature search found XX papers describing spatial and/or temporal variation in the FA composition of organic matter or biota in both lentic and lotic waters.

Spatial variation in the FA profiles in lentic ecosystems

(Li et al. 2018) used FA biomarkers to examine spatial heterogeneity of sedimentary organic matter (SOM) to identify its sources. Higher relative percentages of odd n-alkanes (C26 to C35) and long-chain FA (C24:0 to C32:0) explained the influence of inflow rivers carrying terrestrial inputs on SOM. The higher relative percentages of n-alkanes from C14 to C20, FA (C16:0), and polyunsaturated FA (C18:2 and C18:3) in one lake section demonstrated that some special topology was important for the accumulation of algae-derived OM in sediments. Short-chain and middle-chain biomarker compounds revealed a large contribution from macrophytes in another lake section and an obvious algae-derived organic matter accumulation in an algae-type region, respectively. (Larson et al. 2016) examined secondary production and food quality for consumers across 32 locations in Lake Erie on the border of the USA and Canada. They found secondary production was highest in the more eutrophic sites and lowest in the less eutrophic sites. Mussel tissues in the eutrophic sites also included more eicosapentaenoic and docosapentaenoic fatty acids (EPA and DPA, respectively), but fewer bacterial FAs, suggesting more algae at the base of the food web in the more eutrophic sites. Cyanobacterial abundance among sites was not correlated to secondary production but

was positively related to EPA and DPA content in the mussels, suggesting more of these important FAs in locations with more cyanobacteria.

Temporal or seasonal variation in FA in lentic waters

(Pollero et al. 1981) studied the lipid composition and seasonal changes in the freshwater bivalve *Diplodom patagonicus*. The fatty acid composition of the lipids, remarkably different from that of marine bivalves, and even from other fresh water animals, was especially rich in the ω6 fatty acids, linoleic and arachidonic (ca. 25%), and poor in the ω3 acids, 20:5 and 22:6. The ω6/ω3 acid ratio was approximately two, which is very high compared to marine bivalves. The fatty acid composition and the ω6/ω3 acid ratio were constant during the whole year, suggesting a very stable diet, rich in vegetal detritus and poor in diatoms. The influence of environmental temperature fluctuation with season on fatty acid composition also was negligible. (Goedkoop et al. 2000) quantified FA concentrations and their seasonal variations for profundal benthic invertebrates, surficial sediment, and sedimenting matter from Lake Erken, Sweden. Food quality for profundal zoobenthos, as indicated by the concentrations of long-chain polyunsaturated FA, ω3 FA, or eicosapentaenoic acid (EPA) in sediment and sedimenting matter, was highest in spring and autumn and markedly lower in summer.

(Taipale et al. 2009) investigated the seasonal variation in the diet of Daphnia by analysing the phospholipid fatty acid (PLFA) profiles of seston and of the animals themselves, through the open water period in a small, dystrophic. Algal PUFAs contributed appreciably to total PLFAs in adult Daphnia during spring and summer, but less so in autumn. Biomass of Daphnia in the lake reached their highest biomass in autumn, when methanotrophic activity was also highest, and the highest magnitude of MOB-specific PLFAs was recorded in both adult and juvenile Daphnia. Autumnal mixing evidently stimulates bacterial oxidation of methane from the hypolimnion, and exploitation of the methanotrophic bacteria sustains a high Daphnia population late in the season.

(Roy Chowdhury et al. 2014) determined rates of potential methane oxidation (PMO) and shifts in methanotrophs over hydrological and seasonal gradients. Surface and subsurface soil samples (0-8 or 8-16cm depths) were analyzed for PMO and profiled for methanotroph community structure using phospholipid fatty acid (PLFA) analysis over four seasons (winter, spring, summer and fall) and 3 landscape positions (upland, intermittently flooded, and permanently flooded sites). PMO rates were highest in the winter. PLFA profiling of methanotrophs showed that both Type I and Type II methanotrophs were dominant in winter. PMO and methanotroph biomass were highest in the winter and in the permanently flooded sites which suggested substrate (CH4) concentration was more important in regulating methanotrophy than redox potential or seasonal shifts in temperature under flooded conditions.

(McMeans et al. 2015) investigated how seston fatty acids (FA) and water temperature explained seasonal variation in cladoceran and copepod FA over three years in pre-alpine, oligotrophic Lake Lunz, Austria. Using the mostly algal-derived polyunsaturated FA (PUFA: arachidonic, ARA; eicosapentaenoic, EPA; docosahexaenoic acid, DHA), terrestrial FA (TFA, 22 : 0, 24 : 0), and bacterial FA (BAFA, 15 : 0, 17 : 0 and their branched homologues) as source-specific biomarkers, we show that cladocerans consistently contained more ARA and EPA and copepods more DHA than the available food (seston). None of these physiologically important PUFA were significantly related between zooplankton and seston across the entire study period but copepod DHA increased with seston DHA during the coldest months (< 8°C, based on a significant seston FA*temperature interaction). EPA, conversely, increased with decreasing water temperature in both zooplankton groups. For the nonessential FA, TFA were lower in zooplankton than in seston and not related to dietary supply or water temperature. However, cladoceran and copepod BAFA increased significantly with increasing seston BAFA and decreasing water temperature. These findings suggest that physiological regulation in response to changing water temperature had a significant impact on cladoceran and copepod EPA and the extent of dietary tracking for copepod DHA. TFA available in the seston may not have been consumed or were poorly incorporated by zooplankton, but BAFA were good indicators of available resources throughout multiple seasonal cycles. The authors suggested that both FA type and water temperature impact the extent that dietary vs. nondietary processes govern cladoceran and copepod FA in oligotrophic lakes.

Temporal and spatial variation in FA in lentic systems

(Boon et al. 1996) used phospholipid fatty acids (PLFA) indicative of specific bacterial metabolic groups to identify spatial variation between a permanent and an ephemeral wetland. There were no significant differences in the relative proportions of the sum of branched PLFAs in the sediments between the two sites, across seasons, or between the vegetated and bare zones. Concentrations of total saturated and monounsaturated PLFAs in the sediments followed the same trend as those of the total PLFAs. There was no significant difference in overall composition between the early and late flood periods in terms of total saturated or monounsaturated PLFAs. In contrast, saturated PLFAs were proportionally more abundant in the permanent sediments (approx. 35-45 %) than in sediments from the ephemeral wetland, whereas monounsaturated forms were relatively more common. Gross measures such as total PLFA content, the broad distribution of saturated, monounsaturated and branched fatty acids, and varied little between early and late flooding.

(Hrycik et al. 2018) examined spatial and temporal variation in stomach contents, fatty acids, and stable isotopes of yellow perch (Perca flavescens) across seasons and across sites spanning approximately 200 km in Lake Erie's Central Basin (LECB). They found limited spatial variation of biochemical indicators and more pronounced variation across seasons for all three trophic indicators, especially fatty acid profiles. Fatty acid profiles were predominantly influenced by seasonal fluctuations in C22:6n-3 (DHA). The results suggest that adult yellow perch in LECB showed little differentiation in resource use across space, but their diets and biochemical compositions varied seasonally.

(Lau et al. 2012) analysed the FAs of 22 taxa of benthic macroinvertebrates, zooplankton and fish collected from the littoral, pelagic and profundal habitats of nine boreal oligotrophic lakes over spring, summer and autumn. They quantified and compared the FA variance partitions contributed by species, site and season using partial redundancy analysis both on all consumers and on benthic arthropods alone. Species identity by itself explained between 84.4 and 72.8% of explained FA variation of all consumers and benthic arthropods, respectively. In contrast the factors of site, season and all joint effects accounted for 0–11.3% only. Pelagic and profundal consumers showed stronger reliance on autochthonous resources than did their littoral counterparts as reflected by their higher ω3 to ω6 FA ratios.

Spatial variation in the FA profiles in lotic ecosystems

(Guo et al. 2015) investigated nine streams in South-East Queensland, Australia, to identify environmental factors affecting the fatty acid composition of periphyton. Riparian tree canopy extent and N concentrations explained most of the observed variation in periphyton fatty acid profiles. Nitrogen concentrations showed negative relationships with most saturated fatty acids (SAFA), while mono-unsaturated fatty acids (MUFA) and the fatty acids 16:0 and 16:1ω7 were negatively correlated with canopy cover. In contrast, the percentage of highly unsaturated fatty acids (HUFA) in periphyton was greater with increasing canopy cover regardless of NOx-N concentrations. Variation in riparian canopy cover and nutrients gave rise to opposite outcomes in terms of periphyton food quality and quantity. The highest periphyton food quality, measured by HUFA content, occurred in streams with high canopy cover and low nutrients, while the highest periphyton food quantity occurred in streams with low canopy cover.

(Rude et al. 2016) examined whether differences in fish FA profiles among main channel and connected and disconnected floodplain lakes exist in large river-floodplain systems in the fish Bluegill *Lepomis macrochirus.* Bluegill FA profiles differed among habitats and river reaches, including differences in levels of individual FAs such as 18:2*n*−6 (an indicator of allochthonous inputs), was higher among main channel fish and the *n*−3:*n*−6 FA ratio, an indicator of aquatic primary productivity, was higher among floodplain lake fish. The differences enabled >87.5% reclassification accuracy of fish to their source environment and the authors suggested that FA profiles can be used to infer recent habitat use and habitat-specific foraging of fishes in large river-floodplain ecosystems.

Temporal or seasonal variation in FA in lotic waters

(Sushchik et al. 2007) studied the seasonal composition and concentrations of fatty acids (FAs) in benthos from pebbly littoral region of the Yenisei River in a sampling site near Krasnoyarsk city (Siberia, Russia). They concentrated on major long-chain polyunsaturated fatty acids (PUFAs) of the ω3 family: eicosapentaenoic acid (EPA, 20:5ω3) and docosahexaenoic acids (DHA, 22:6ω3). In the diatom dominated phytobenthos the annual maxima of EPA and DHA pool occurred in spring and early summer. In the

zoobenthos, EPA and DHA pool peaked in autumn, due mainly to an increase of the biomass of gammarids and to a moderate increase of the PUFA content per body weight. Seasonal peaks of EPA in overwintering insect larvae (chironomids and caddisflies) generally coincided with those of biomass of these larvae, while there was no such trend for amphipods and oligochaetes. In spring and early summer, the main part of ω3 PUFA, 40–97% of total amount, in the littoral region was contained in biomass of producers, i.e., benthic microalgae, and in autumn it was transferred to primary consumers—benthic invertebrates, which contained ∼76–93% of total ω3 PUFAs.

(Twining et al. 2017) documented foodweb structure and examined FA composition as a metric of food quality in an Adirondack stream throughout the temperate growing season. In spite of major seasonal shifts in environmental factors, such as light availability and temperature, they found limited seasonal variation in the FA composition of basal resources and macroinvertebrates. Instead, we found consistent differences in FA composition between aquatic and terrestrial basal resources and between macroinvertebrate functional feeding groups.

Temporal and spatial variation in FA in lentic systems

(Honeyfield and Maloney 2015) examined seasonal patterns in periphyton FA in six minimally disturbed headwater streams in Pennsylvania's Appalachian Mountains, USA. Environmental data and periphyton were collected across four seasons for fatty acid and algal taxa content. The authors observed significant seasonal differences in fatty acids. Summer and fall fatty acid profiles were driven by temperature, overstory cover, and conductivity and winter profiles by measures of stream size. The physiologically important fatty acid 18:3ω6 was highest in summer and fall. Winter samples had the highest 20:3ω3. Six saturated fatty acids differed among the seasons. In contrast, the authors found very little differences in FA among streams.

(Carassou et al. 2017) investigated ontogenetic and seasonal variations in the diet of the freshwater mullet (*Myxus capensis*) across a river–estuary interface using FA dietary tracers. They tested two hypotheses were tested. Firstly, the freshwater mullet diet shifts as individuals grow and migrate from the estuary to the river. Secondly, the dominant food resources utilized by freshwater mullet vary through time, mainly as a function of the seasonal changes in the availability of preferred food items in each habitat. Both hypotheses were supported, as our results indicated broad dietary flexibility by M. capensis, with utilized food items ranging from benthic microalgae to insects depending on habitat and seasonal patterns in availability of resources.

Fatty acids as biomarkers and qualitative and quantitative studies

The literature review recognised 11% of FA papers in freshwater ecosystems were primarily concerned in using them as biomarkers to identify basal food sources, e.g. bacterial or green algae etc. (Table 1). Some lipid species, including FA, are limited to or are synthesised by certain taxa, so if the FA in question is metabolically stable (or retains its basic structure after consumption) it may be used to trace energy transfers through a food web, thus helping to define predatory-prey relationships (Napolitano 1999). Examples of FA specific to particular biotic groups include the bacteria-specific FA isoseptadecanoic acid (17:0iso) (Goedkoop et al. 2000), fatty acids with more than 24 carbons for terrestrial plants (Falk-Petersen et al. 2002) and 18:1n 9 for brown algae (Johns et al. 1979). 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 18:3ω6 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 PLFA 20:5x3 although an often consistent biomarker for diatoms is often not found in large quantities relative to other PLFA (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).

The use of FA as biomarkers to identify basal food sources is a qualitative analysis, the FA indicate potential food sources but they do not quantify the amount or percentages of different food sources supporting consumers. Quantitative fatty acid signature analysis (QFASA (Iverson et al. 2004)) has become a popular method of estimating diet composition, especially for marine species (Bromaghin et al. 2017). A primary assumption of QFASA is that constants named calibration coefficients, which account for the differential metabolism of individual fatty acids, are known. In practice, however, calibration coefficients are not known, but rather have been estimated in feeding trials with captive animals of a limited number of model species. The impossibility of verifying the accuracy of feeding trial derived calibration coefficients to estimate the diets of wild animals is a foundational problem with QFASA that has generated considerable criticism (Bromaghin 2017). The literature search only identified one paper that had attempted QFASA in a freshwater environment. (Taipale et al. 2016b) used FA based modelling of field collected cladocerans to generate estimates of dietary resource assimilation by zooplankton of different basal resources. The calibration coefficients used in the modelling consisting of known FA profiles of Daphnia fed a diversity of known basal monocultures in controlled laboratory feeding trials. The authors used the Bayesian mixing model FASTAR (Galloway et al. 2015) to estimate the basal food sources of field caught animals in lake ecosystems. A more novel modelling technique has been developed which simultaneous estimation of diet composition and calibration coefficients based only on fatty acid signature samples from wild predators and potential prey (Bromaghin 2017, Bromaghin et al. 2017). This modelling technique has been developed for marine species but represents a potentially valuable research tool for both lentic and lotic ecosystems in future.

Extra research

- Climate change using multiple consumer levels
- Invasive species in running waters
- Ephemeral or intermittent streams – subsidy terrestrial aquatic
- Other anthropogenic impacts, particularly river regulation and colmation.
- Spatial and temporal of primary producers and consumers in lentic and lotic environments.
- Freshwater quantitative fatty acid signature analysis

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Appendix 1: Research Activity Reports

Appendix 1.3 Basal resource transfer efficiency between a range of basal resources and to first-order consumers (mesocosm experiments)

1.3.3 Patterns of invertebrate emergence and succession in flooded wetland mesocosms.

Keywords: wetland, mesocosm, invertebrate, Murray Darling Basin

Abstract

Floodplain wetlands are some of the most productive ecosystems available to aquatic and terrestrial organisms. However, regulation of lowland rivers can disrupt ecological processes occurring in the river-floodplain ecosystems and environmental water can be delivered to affected wetlands to maintain productivity. It is not well understood at what stage following inundation there would be sufficient invertebrate biomass and large sized individuals to support production and reproduction of secondary consumers. In this study we follow changes in the abundances of invertebrates after wetting in three mesocosm trials using soil from two wetlands from the northern Murray Darling Basin over six-week periods. The patterns of changes in invertebrate abundances differed between taxa, wetland soils and the different trials. In general, abundances increased between the second and fourth weeks and then either declined or continued to increase. Peak abundances generally occurred in either the fifth or sixth weeks. Our results suggest that inundation of wetlands using environmental watering can achieve high productivity within a short time frame, within weeks. However, how quickly the high productivity is passed onto second order consumers remains unclear and should be an area of future research.

Introduction

Floodplain wetlands are some of the most highly productive ecosystems available to aquatic and terrestrial organisms (Opperman et al. 2010). They play a significant part in river function through nutrient cycling, storage of sediments and water and supporting substantial primary production and high biodiversity (Junk, Bayley, and Sparks 1989; Tockner, Malard, and Ward 2000). In arid-zone rivers largely unaffected by water resources development, inundation of floodplains can result in increases in autotrophic communities and metabolism (Bunn et al. 2006; Costelloe et al. 2005), germination of aquatic macrophytes (Capon and Brock 2006), invertebrate emergence from soil egg banks and insect colonisation (Maher 1984; Shiel et al. 2006), proliferation of fish populations (Puckridge et al. 1998) and the gathering of large numbers of waterbirds (Kingsford, Curtin, and Porter 1999). However, regulation of lowland rivers usually alters many characteristics of the flooding regime including frequency duration and extent of inundation of their floodplains (Boulton and Lloyd 1992). The impacts of altered hydrology can disrupt ecological processes occurring in the river-floodplain ecosystems because connectivity is mediated by flows and hydrological linkages (Jenkins and Boulton 2003). The link between altered wetland hydrology and the effects on biota is exemplified in the Murray Darling Basin where increases in water extraction for irrigation have resulted in significant declines in shorebird populations (Nebel, Porter, and Kingsford 2008).

Invertebrates play a critical role in food webs (Boon and Shiel 1990), linking aquatic and terrestrial carbon sources to higher trophic levels such as fish and waterbirds (Lindholm and Hessen 2007). Invertebrates hatching from egg banks stored amongst the sediments or imported from the river (Jenkins and Boulton 2003) can comprise a significant proportion of the biomass in wetlands (Crome and Carpenter 1988). The density of invertebrates and taxonomic structure differs both spatially and temporally amongst wetlands. Densities of over 30,000 animals per litre have been recorded in Australia (Kobayashi et al. 2015) but much smaller densities are more typical e.g. (Boulton and Lloyd 1992; Crome and Carpenter 1988). The actual biomass and/or invertebrate abundance available to fish and birds will depend on their size; the size of invertebrate prey increases with fish gape size while fish larvae will also eat smaller animals (King 2005). However, very small invertebrates that cannot be seen by predators are unlikely to be prey. In some wetlands the invertebrate taxa are dominated by rotifers e.g. (Shiel et al. 2006) but other wetlands can be dominated by microcrustaceans such as cladocerans and ostracods e.g. (Górski et al. 2013). While each of

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these groups have been identified as prey, microcrustaceans appear to be preferred over rotifers (Kaminskas and Humphries 2009; King 2005).

Following the inundation of a wetland there is a gradual increase in invertebrate density and biomass both through emergence from soil egg banks and recruitment through breeding in different habitat types (Boulton and Lloyd 1992; Jenkins and Boulton 2003). However, the timing of the emergence differs between invertebrate taxa. Often nematodes can be found only after a day following inundation but other groups, notably ostracods, may only be found after a week (Boulton and Lloyd 1992). It is not well understood at what stage following inundation there would be sufficient invertebrate biomass and large sized individuals to support production and reproduction of secondary consumers. However, Lindholm and Hessen (2007) suggest there is high primary and secondary production in wetlands within the first few weeks of wetted floodplains. The majority of studies that have observed short-term invertebrate succession following wetting of wetland soils have taken place in mesocosm experiments e.g. (Boulton and Lloyd 1992; Brock, Nielsen, and Crossle 2005; Jenkins and Boulton 2003) rather than in natural wetlands, possibly due to logistical issues and lack of resources. In this study we followed changes in the abundances of invertebrates after wetting in three mesocosm trials using soil from two wetlands from the north of the Murray Darling Basin over six-week periods. We hypothesised that the abundances of invertebrates would increase following inundation and that the patterns would be similar between the separate trials and between wetlands.

Methods

Study wetlands

The Gwydir wetlands are located in the Gwydir catchment, part of the Murray-Darling Basin in northwest NSW. These terminal wetlands form part of the Gwydir River (catchment area of 25 900 km^2), which flows from the New England Plateau in the east to the Barwon River at Collarenebri in the west (Office of Environment and Heritage 2011). All major tributaries join the Gwydir River upstream of Moree, while downstream the channels form an inland delta of extensive floodplains. Downstream of Moree the river divides into two floodplains, the Gingham Channel to the north and the Gwydir Channel to the south. The principal land use on these floodplains and wetlands is private grazing and cropping and the area experiences highly irregular flooding regimes. In all but the largest floods, flows are held in the wetlands and floodplains and do not contribute to flows in the Barwon River.

The Gwydir River system is highly regulated by an extensive series of dams, weirs and diversion channels. Flows into the wetlands are largely regulated by the largest of these, Copeton Dam upstream of Moree. Artificial watering of the wetlands occurs via environmental flows which are released from Copeton. The volumes and timing of the artificial flows are determined by the New South Wales and Federal governments under the direction of the Murray-Darling Basin Authority Basin Plan's environmental watering plan (Murray–Darling Basin Authority 2014).

Mesocosms set up

Dry wetland sediment to a depth of approximately 10 mm was collected from the two nationally and internationally important wetlands (Office of Environment and Heritage 2011), the Bunnor property on the Gingham watercourse and the Old Dromana (OD) property on the Gwydir watercourse (29°16'S 149°21'E and 29°20'S 149°20'E, respectively) in April and September 2017 and March in 2018. The three collection dates were to provide soil for three different trials of emergence following mesocosm flooding. The vegetation on the Bunnor and Old Dromana sites is classified as water couch marsh grassland wetland of frequently flooded inland watercourses (Bowen and Simpson 2010; Southwell et al. 2015). Sediment was put through a sieve to separate soil from roots and vegetation. Sieved sediment from each wetland (350 g) from each collection date was placed in each of 18 4L containers (200 x 200 x 120 mm) and filled with 2L of rainwater and maintained at that volume throughout the experiment. The mesocosms were kept at a constant 24°C with 24 hr lighting and gently aerated through the course of the experiment. Individual shield shrimp (Notostraca: *Triops australiensis*) were removed from the containers when observed as a pilot study and Boulton and Lloyd (1992) had shown that either their activity within the sediment or their feeding behaviour reduced the populations of other invertebrates.

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Invertebrate sampling and processing

Three containers with each wetland soil were sampled at weekly intervals for six weeks in total. For each container the supernatant was poured through a 63 μm sieve, the material preserved in 70% ethanol and stained with rose bengal. The remaining soil was agitated and flooded to a depth of 60 mm and let stand for 5 min. The material from resulting supernatant was again sieved, preserved and stained. Each sample was sieved through a 250 and 63 μm mesh. The 250 μm fraction was chosen to represent potential prey to secondary order consumers and the 63 μm fraction chosen to represent future potential food but also to generate information about small animal groups, mainly nematodes and rotifers. All invertebrates collected in the 250 μm sieve were identified and counted. Invertebrates in the smaller sieve size were subsampled if very abundant by taking 1 ml aliquots from the material suspended in 20 ml of ethanol and identifying and counting till over 100 animals had been processed. Abundances were then calculated by multiplying the subsample abundance by the reciprocal of the proportion of sample processed. Cladocerans were identified to family level, ostracods to class, copepods, conchostracans, arachnids and rotifers to order, tardigrades and nematodes to phyla and notostracans to species. The total abundance of each of the taxonomic groups for each of the size fractions is reported as the number of animals per litre.

Results

A total of 356,665 invertebrates were processed from ten taxa. The majority of the animals were the microcrustaceans Macrothricidae (59%) and Chydoridae (24%) followed by Rotifera (10%), Ostracoda (5%) and Nematoda (1%). In contrast, fewer than 20 individuals of Calanoida, Conchostraca, Notostraca, Sarcoptiformes and Tardigrade were processed. The abundances of all taxa were greater in the 63 μm size fraction compared with the 250 μm fraction (Figure 1 and Figure 2). No rotifers were present in the 250 μm fraction during any of the trials.

The patterns of changes in abundances differed between taxa, wetland soils and the different trials. In general, abundances increased between the second and fourth weeks and then either declined or continued to increase. Peak abundances generally occurred in either the fifth or sixth weeks. Nematoda were present in both wetland soils within one week of wetting. In contrast, the other taxonomic groups generally appeared in the second week of inundation.

Discussion

We demonstrated that the soils of the Bunnor and Old Dromana wetlands contain eggs or dormant stages of ten taxa. The timing of the appearance of the different taxa is similar to other wetland mesocosm experiments, with Nematoda generally appearing first followed by the other taxa. Boulton and Lloyd (1992) suggested that the reason for the early occurrence of Nematoda was that they were probably already active in dry sediments prior to flooding. Large numbers of invertebrates, particularly microcrustaceans, were present in the mesocosms generally after two to three weeks following inundation. The pattern of emergence was similar between the two size fractions indicating that there is potentially enough invertebrate prey to support secondary consumers within a short time of flooding. The actual number of prey or their densities required for the survival and maintenance of larval fish in Australian freshwater systems is not currently known (King 2004). However, Geddes and Puckridge (1989) demonstrated that there are sufficient resting stages of invertebrates to support fish larval populations in one month in earthen ponds.

The increase in abundance of invertebrates in our wetland mesocosms took place without external supplies of nutrients. This is also the case for other mesocosm experiments carried out using soil from wetlands in the Murray Darling Basin (Boulton and Lloyd 1992; Brock, Nielsen, and Crossle 2005; Hay, Jenkins, and Kingsford 2018; Jenkins and Boulton 2003). This generally indicates dry wetland soil contains enough sources of carbon and other nutrients to support both invertebrate populations and secondary consumers. However, the source of the carbon, e.g. from bacteria, algae etc., remains unclear. Although we have demonstrated that dry wetland soil can support ecosystem function, in natural systems flooding from connecting rivers would undoubtedly bring additional carbon and nutrients. The importance of each source of carbon and nutrients remains unclear. However, such information would be useful for determining

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mechanistic processes underlying wetland functions and would allow a more thorough process-based evaluation of environmental water used in the Murray Darling Basin (Robson et al. 2017; Rolls et al. 2017).

Although we demonstrated the emergence and subsequent proliferation of invertebrates from the dry soil, there was considerable variability in the succession patterns among the different taxa and between wetlands. The variability we observed may reflect patchiness in microtopography where the wetland soil was collected and resting stage settlement when the wetlands dried out (Boulton and Lloyd 1992). There is also variation the abundances among taxa and differences in the taxa collected from the mesocosms compared with taxa collected from the wetlands when they have been inundated. Between 17 and 20 taxa have been collected from the wetlands (W. Tsoi, unpublished data) compared with the ten taxa collected during the present study. Nevertheless, mesocosm experiments provide an important bridge between smaller, more tightly controlled, microcosm experiments (which can suffer from limited realism) and the greater biological complexity of natural systems (in which mechanistic relationships often cannot be easily identified) (Stewart et al. 2013). So despite the potential limitations of our experiment and the successional variability demonstrated our results suggest that inundation of wetlands using environmental watering can achieve high productivity within a short time frame, within weeks. However, how quickly the high productivity is passed onto second order consumers remains unclear and should be an area of future research.

Acknowledgements

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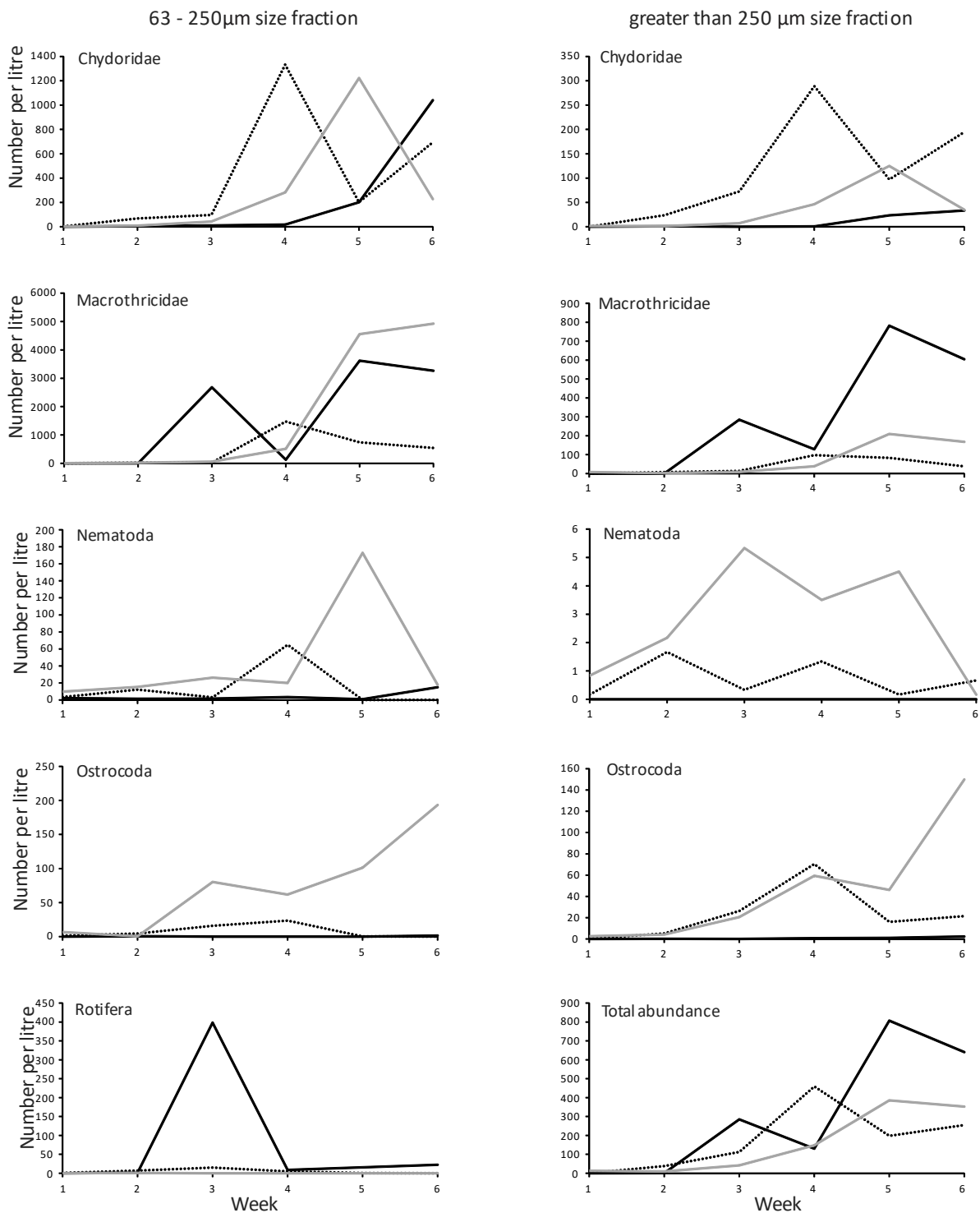
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Figure 1. Pooled abundances of different taxa and total abundances in three trials for two sizes of invertebrates from the Old Dromana wetland soil. Black lines represent invertebrates from the first trial run, dotted lines the second trial run and gray lines the third trial run.
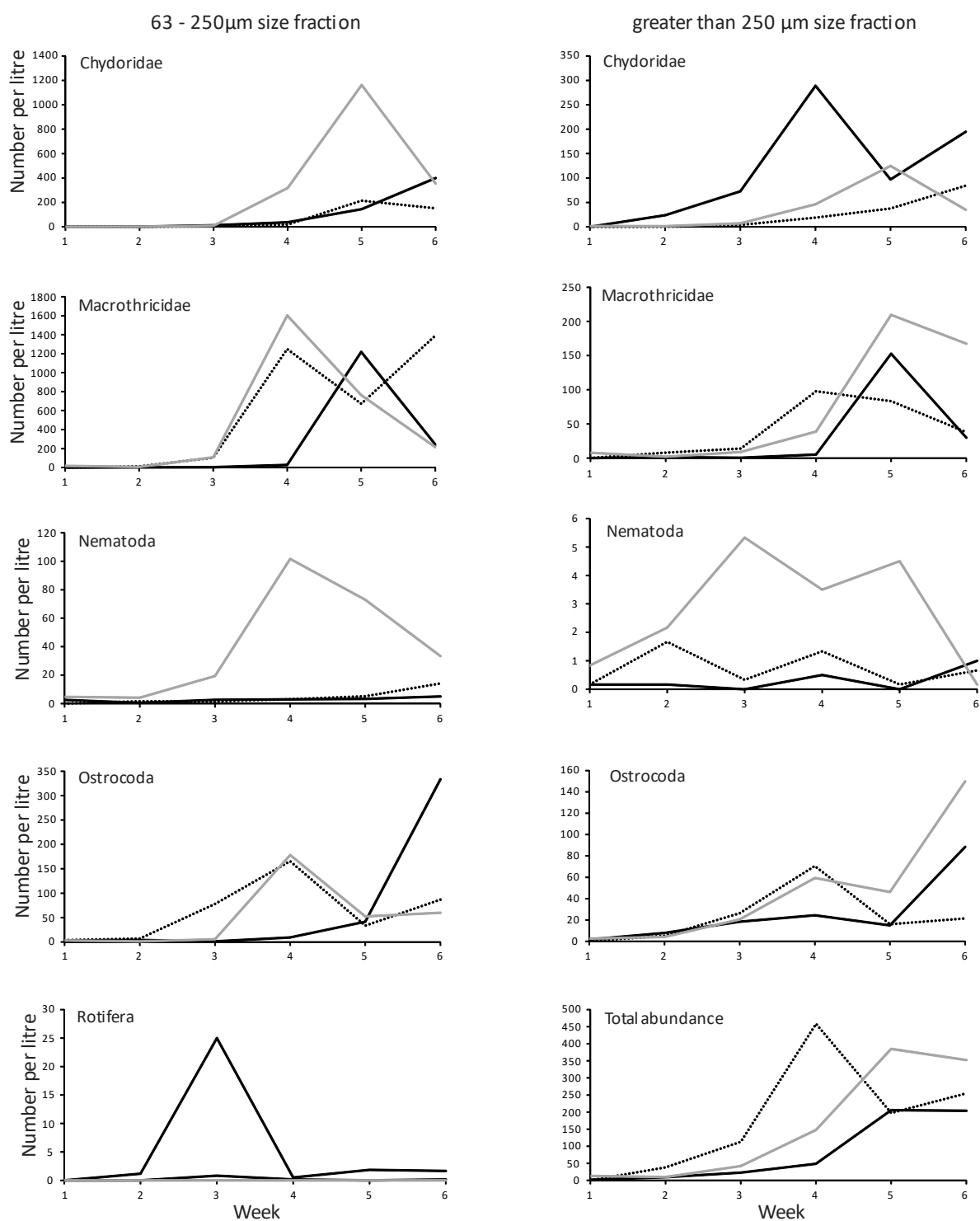
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Figure 2. Pooled abundances of different taxa and total abundances in three trials for two sizes of invertebrates from the Bunnor wetland soil. Black lines represent invertebrates from the first trial run, dotted lines the second trial run and grey lines the third trial run.

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Appendix 1: Research Activity Reports

Appendix 1.4 Basin scale trophic niche indicator

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Abstract

Food and energy availability is one of the most important factors determining fish recruitment in rivers and is vital to sustain adult growth and reproduction. Here we evaluate how the stable isotope trophic niche (δ13C and δ15N) of fishes varies among species, trophic functional guilds, body sizes, and rivers (LTIM selected areas) where environmental flows are being delivered and monitored in the Murray-Darling. The focus was on large-bodied Murray cod and golden perch which are considered top predators in rivers of the Murray-Darling and therefore provide a measure of Food Chain Length (FCL). The maximum trophic position (TP) and therefore FCL, ranged from 3.82 to 4.54, and in all rivers was consistently golden perch, not Murray cod. This result contrasts with anecdote and previous gut content analyses suggesting that Murray cod are the apex predator of rivers in the Murray-Darling. The most parsimonious model identified significant variation in TP among rivers irrespective of species and length, although TP also increased significantly with body length in Murray cod, golden perch and common carp. The diversity of δ13C signatures supplying fishes also varied significantly among species, body lengths and among rivers where environmental flows are being delivered. The trophic niche indicator developed here provides a monitoring tool for managers to evaluate changes in the trophic position and diversity of carbon sources fuelling top predators and fish communities in the Murray-Darling. Future monitoring of trophic niche would allow managers to assess whether the delivery environmental flows are changing the food resources supplying fish populations and how, or if flow deliveries, are benefiting fish recruitment and food web structure leading to more productive populations of top predators.

Introduction

Food and energy availability can limit recruitment (Humphries et al. In-press) and the production of consumers and top predators (e.g. fish, waterbirds), yet these constrains have only recently been considered in developing restoration and management strategies for river ecosystems (Naiman et al. 2012). Trophic niche is a measure of the food resources and energy supplying consumers and is a fundamental dimension of food web structure (Layman et al. 2007). It is determined by energy availability, energy transfer and the diversity of Carbon and nutrients in ecosystems. Ratios of nitrogen and carbon stable isotopes (Post 2002) from consumer tissues, such as fish (Jardine et al. 2013; Ou et al. 2017), provide a representation of 'trophic niche' (Layman et al. 2007), and can be evaluated quantitatively to assess ecological changes, including responses to management or restoration actions including environmental flows.

When stable isotope ratios of nitrogen are estimated for a top predator (e.g. Murray cod), the trophic position provides an estimate of how high in a food web that a consumer feeds and it is a measure of food chain length (Post 2002). Food chain length (FCL) has been correlated with river flows in that higher discharge often creates more food chain links which support higher level consumers, such as waterbirds and fish (Sabo et al. 2010). Conversely, some highly productive tropical river ecosystems and fish communities are supported by short, highly productive, food chains (Winemiller 1990; 2004). Since river flows are drivers of energy production and influence how different sources of energy enter food webs (Humphries et al. 2014; Thorp & Bowes 2017), it is important to develop a better understanding of how best to manage river flows and ecosystems to maximise the food and energy resources available to native consumers (Naiman et al. 2012).

The aim of this study was to develop an indicator of the trophic niche of Murray-Darling Basin fishes in collaboration with long-term intervention monitoring (LTIM) of environmental flows and the environmental water knowledge and research (EWKR) project. The research objectives were to evaluate how trophic niche ($\delta13C$ and $\delta15N$) of fishes varies among species, trophic functional guilds, body sizes, and rivers (LTIM selected areas) where environmental flows are being delivered and monitored in the Murray-Darling. The focus was on large-bodied Murray cod and Golden Perch which are considered top predators (Ebner 2006) in rivers of the Murray-Darling.

Methods

Field sampling

We collected tissues from fish, basal resources and water samples for Chlorophyll a (Chlor a) and Dissolved Organic Carbon (DOC) between March and May 2018 in the Edward-Wakool, Goulburn, Lachlan, Lower Murray, and Murrumbidgee rivers. Fishes were sampled using standardised boat-electrofishing and fyke netting carried out as part of LTIM category I monitoring. Briefly, fish were sampled at 10 sites, spanning total reach lengths of 20-100 km, in each river (Figure 1). Each site was sampled once annually using 10 fyke nets and 2880 s of electrofishing 'on time'. Fish were identified and the length of the first 40 individuals of each species per site was measured (mm). A caudal fin-clip (30-50 mm^2) was collected from up to 30 individuals, per species per river, for fish larger than 150 mm, while fish smaller than 150 mm were euthanized to ensure a sufficient quantity of tissue for stable isotope analyses. Fin-clips from an addional 7 Murray cod ranging from 600 mm to 1100 mm TL sampled from the Mid-Murray were included in analyses. Fin-clips were stored in 1.5 mL non-stick centrifuge tubes and whole fish in plastic zip-lock bags on ice and then frozen before preparation for stable isotope analyses.

Five basal resources were sampled at two sites in the upper, middle and lower reach of each river (Figure 1) during the period of fish sampling activities in order to provide a baseline $\delta15N$ for trophic position estimates. At each site, the basal resources sampled included: 1) periphyton (benthic algae, filamentous algae and associated biofilm); 2) seston (phytoplankton and suspended fine particulate matter); 3) macrophytes; 4) grasses and 5) terrestrial riparian plants. Periphyton was scraped from woody debris, rocks and macrophytes using scouring pads and placed in zip-lock bags. Seston was collected from epilimnetic water samples in 1 L bottles prefiltered through 250-μm mesh and then filtered onto precombusted Whatman GF/F filters (pore size 0.7 um). Macrophytes, grasses and plant leaves, seeds and stems were collected from a mixed variety of species visually assessed within a 500 m reach at each site, cut into small pieces, placed into plastic zip-lock bags and frozen.

Stable isotope and laboratory analyses

Frozen basal resources, fin-clips and whole small-bodied fish were thawed and rinsed (excluding seston filter papers) with reverse osmosis purified water and dried in glass vials or petri dishes in an oven at 60°C for 48 h (Arrington and Winemiller 2002). Fin-clips from 10-15 individuals of each species of piscivore and omnivore and 7 individuals of each species of insectivore-microcrustacivore and algivore-detritivore were selected from each river and processed for bulk stable isotope ratios of nitrogen ($^{15}N/^{14}N$) and carbon ($^{13}C/^{12}C$) at the University of Western Australia. Samples were analysed for $\delta15N$ and $\delta13C$, using a continuous flow system consisting of a Delta V Plus mass spectrometer connected with a Thermo Flush 1112 via Conflo IV (Thermo-Finnigan/Germany). The isotope values $\delta15N$ and $\delta13C$ are reported in relation to [‰, Air] and [‰, VPDB] respectively according to international standards (Skrzpek 2013)

Fifteen species of fish were sampled for stable isotope analyses and individuals spanned a wide range of lengths from 18 mm to 1100 mm (Table 1). Individual fish selected for stable isotope analyses reflected the range and mean length of each species sampled within each river. Some species were not present, or sufficiently abundant, in all rivers to fulfil sample size requirements and in these cases all individuals were processed for stable isotope analyses. The larger sample size of piscivorous and omnivorous fish species was necessary to effectively evaluate the wider range of lengths and greater variation in trophic niche observed across their size range and ontogeny.

Chlor a and DOC samples were carried out in a CFE NATA accredited analytical laboratory. Chlorophyll pigments were extracted in hot 90% (v/v) ethanol (5 min at 75 °C). Chlor a was determined by spectrophotometric absorption and concentrations calculated as µg l-1. Determinations of DOC were performed by wet oxidation using a model 1010 wet oxidation TOC analyzer by OI Analytical.

Trophic guilds and trophic position

Fishes were classified into four trophic guilds based on a diet analysis of Australian freshwater fishes by Stoffels (2013) and references therein. The trophic guilds align with internationally recognized descriptions by Ou et al. (2017): 1) piscivore: fish that primarily consume fish and in some cases decapod crustaceans and other macroinvertebrates; 2) omnivore: trophic generalists that consume a range of phytoplankton, benthic algae and aquatic or terrestrial invertebrates; 3) algivore-detritivore: fish that consume detritus or algea; 4) insectivore-microcrustacivore: fish with diets dominated by insects, aquatic microcrustaceans or other invertebrates. We combined the guilds 'microcrustacivores' and 'aquatic insectivores' defined by Stoffels (2013) since some species sampled in our study could not be clearly distinguished between the two guilds.

The trophic position (TP) of fishes was calculated according the equation (Post 2002; Vander Zanden and Rassmussen 1999): TP = δ15N $_{consumer}$ - δ15N $_{basal\ source}$) / 3.4 +1, where δ15N $_{consumer}$ is the signature of individual fish, and δ15N $_{basal\ source}$ is the mean δ15N of the basal resources sampled within each river where fish were sampled. The value 3.4 represents the shift in nitrogen between its ingestion by a consumer and its assimilation into the consumer's tissue.

Statistics

Statistical analyses here focused Murray cod and golden perch which are considered top predators (Ebner 2006) in the Murray-Darling Basin and were evenly sampled with 10-15 individual fish sampled per river across the five LTIM selected areas. Common carp were also included in analyses and were evenly sampled with 10-15 individuals per river.

Generalized Linear Mixed Effects Models (GLMMs) were used to examine how δ15N and δ13C signatures of the five basal resources varied among types and rivers using the equation: δ15N or δ13C ~ basal resource type + river + basal resource type:river, where basal resource type was seston, macrophytes, periphyton, grasses, or terrestrial riparian plants and river was the Edward-Wakool, Goulburn, Gwydir, Lachlan, Lower-Murray or the Murrumbidgee.

GLMMs were also used to evaluate how the TP (δ15N baseline corrected) of fish and their δ13C signatures varied in relation to species, functional guild, length, and river according to the equations: TP (δ15N baseline corrected) or δ13C ~ loglength + species + loglength:species + river + functional guild. Species and functional guild were correlated and therefore examined in separate models.

Sites were modelled as random effect (1/site) in all GLMM's. We fitted the full models above and all possible combinations of variables, with and without interactions, and ranked them according to the lowest Akaike Information Criterion (AIC). Pseudo r^2 values were calculated as a measure of goodness of fit (Nakagawa and Schielzeth 2013). A difference of 2 or more between AIC values was used to distinguish important differences among models. We assumed that all coefficients not overlapping zero (null model) were important predictors.

Bi-plots of TP (δ15N baseline corrected) and δ13C were used to illustrate the

trophic niche of Murray cod, golden perch and common carp among rivers and niche metrics calculated included the minimum and maximum TP (FCL) and range of δ13C observed in each river. Mean, or central

tendency estimates of TP and δ13C were not reported since GLMM results better described how they co-vary in relation to species, length and river. The maximum trophic position (Post 2002) observed (either Murray cod or golden perch) in each river was used to represent the food chain length (FCL). The mean DOC and Chlor a concentration of each river was calculated from six replicate sites and used to inform the discussion and interpretation of stable isotope results.

Results/Discussion

The trophic position of Murray cod, golden perch and common carp all increased significantly and positively with length up to a maximum of 4.5 (Figure 2). Interestingly, the maximum trophic position and therefore FCL (Table 2) in all rivers was consistently golden perch, not Murray cod. This result was not due to differences in length and contrasts with anecdote and gut content analyses (Ebner 2006) suggesting that Murray cod are the apex predator of rivers in the Murray-Darling. Our sample here was a single season isotopic snap-shot of diet, but covered five major rivers in the lower Murray-Darling. The wide range of variation in Murray cod TP suggests that this species is more omnivorous than previously assumed and that golden perch may be more piscivorous. Indeed, across small to medium size ranges (Figure 2), omnivorous common carp shared a similar trophic position to Murray cod.

The trophic position of fish was explained best by models incorporating species identity, rather than functional guild (best model AIC = 327), and an interaction between species x loglength (Table 3, model 4; Table 4). These results suggest that trophic position increases differently for each species and therefore size-based food web models which often assume a common positive allometric scaling exponent (e.g. Romanuk et al. 2011) will be less reliable when applied to the species examined here. Our results suggest a complex relationship between species length and trophic position and is consistent with studies on other freshwater fishes and river systems showing that trophic position does not always increase similarly with increasing body size (Ou et al. 2017).

The most parsimonious model (Table 3, model 4) also identified significant variation in TP among rivers irrespective of species and length. This indicates that ecological or anthropogenic factors other than those included in the models here may explain TP. Given that this was a single year study it was not possible to evaluate alternative hypotheses regarding the effects of rivers flows on TP. However, multi-year datasets combined with calculating flow metrics for a wider range of rivers would allow tests to explain the effects of flow on TP and FCL guided by hypotheses related to ecosystem size, productivity and stability (Post 2002) but converted to river flow metrics (e.g. Sabo et al. 2010).

The trophic carbon niche of fishes (Figure 3) showed a less depleted δ13C signature in the Lower Murray and Lachlan rivers generally indicative of periphyton and seston signatures (Figure 4), whereas other rivers showed more depleted carbon signatures often related riparian or terrestrial plant sources (Jardine et al. 2013; Ou and Winemiller 2016). The δ13C signatures of fish varied significantly among species, length and among rivers (Table 3, model 1; Table 5). However, DOC concentrations were highest in the Lower Murray and Lachlan Rivers (Table 6).

The significant length- δ13C relationships (Table 5) for common carp and strong relationship for Murray cod suggests that the carbon sources supporting individuals (within the juvenile to adult size ranges sampled) varied depending on their size and presumably age and ontogenetic stage. The positive relationships are consistent with hypotheses suggesting that riparian or terrestrial plant sources contribute more to the diet of small and young fish of both species, whereas larger adults may be supported by periphyton and seston production.

The types of basal resources sampled varied significantly in their δ13C isotopic signatures and with their interactions among rivers (Figure 4) according to the most parsimonious model (AIC = 871; pseudo r2 = 0.68; δ13C ~ basal resource type + basal resource type:river). Given the unique δ13C signatures of basal resources, a stable isotope mixing model could be applied to distinguish relative proportions of carbon contributing to fish diets and over time (given future monitoring).

Management implications

The trophic niche indicator developed here provides a monitoring tool for managers to evaluate changes in the trophic position and diversity of carbon sources fuelling top predators and fish communities in the

Murray-Darling. It provides a cost-effective annual monitoring tool relevant to CEWO watering objective 'ecosystem function' and The Basin Plan 'to protect and restore the ecosystem functions of water-dependent ecosystems' (Basin Plan, Chapter 8, Part 2, 8.04(b)).

The trophic position of the fish community is expected to increase if there are more large native predators in the system and will decrease if there are more invasive common carp. It also provides a measure of the food sources and their diversity supplying native fishes. Given multi-year datasets, the trophic niche indicator could be developed to evaluate the direct effects of flow metrics (e.g. Sabo et al. 2010), and therefore environmental flows, on the trophic niche of fishes and other consumers including waterbirds.

What the trophic niche indicator tells us that we won't know from existing LTIM of environmental flows in the Murray-Darling is whether or not the delivery of flows is providing the food resources needed for recruitment and for maximising the production of adult fish populations. In rivers where adult fish are spawning, it remains uncertain why recruitment may not be happening and why adult populations may not be recovering.

Food - is one of the most important factors determining fish recruitment in rivers (Humphries et al. In-press) and is vital to sustain adult growth and reproduction. Therefore, managers need to develop strategies for environmental flows which supply the necessary food resources, at the right times, for adult spawning and subsequent recruitment. Using the trophic niche indicator managers could assess whether the delivery environmental flows are changing the food resources suppling fish populations and how, or if flow deliveries, are benefiting fish recruitment and food web structure leading to more productive populations of top predators.

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Table 1. Trophic functional guilds and length (mm) descriptive statistics for fish species sampled for stable isotopes (δ13C and δ15N) analyses.

Species	Functional Guild	n	Mean	±	Std.	Min.	Max.
B. bidyanus	Omnivore	19	234	±	36	151	284
C. auratus	Omnivore	20	111	±	53	59	220
C. carpio[1]	Omnivore	65	373	±	165	46	627
C. stercusmuscarum fulvus	Insectivore-microcrustacivore	23	40	±	11	25	70
G. holbrooki	Insectivore-microcrustacivore	28	32	±	6	23	46
Hypseleotris spp.	Insectivore-microcrustacivore	28	33	±	7	20	49
M. ambigua[1]	Piscivore	63	424	±	55	294	530
M. fluviatilis	Insectivore-microcrustacivore	28	52	±	19	24	94
M. peelii[1]	Piscivore	71	446	±	274	50	1100
N. erebi	Algivore-detritivore	36	127	±	67	33	293
P. fluviatilis	Piscivore	1	152	±		152	152
P. grandiceps	Insectivore-microcrustacivore	21	37	±	10	20	58
P. macrostomus	Insectivore-microcrustacivore	16	31	±	7	18	40
R. semoni	Insectivore-microcrustacivore	29	46	±	6	36	61
T. tandanus	Omnivore	5	471	±	24	437	493

[1] Denotes species used in statistical models examining variation in δ13C and δ15N.

Table 2. Trophic niche metrics for Murray cod (M. peelii), golden perch (M. ambigua) and common carp (C. carpio) sampled in the Edward Wakool (EW), Goulburn (GB), Lachlan (LC), Lower Murray (LM), and Murrumbidgee (MB). Trophic position estimates calculated from δ15N baseline corrected values and Carbon range from Min. – Max. δ13C values.

Species	Trophic niche metric	EW	GB	LC	LM	MB
M. peelii	Min. trophic position	2.34	3.17	2.19	2.58	3.23
	Max. trophic position	3.23	3.85	3.06	3.19	4.19
	Carbon range	-3.29	-3.00	-5.12	-2.40	-7.36
M. ambigua	Min. trophic position	3.46	3.69	3.16	3.42	3.89
	Max. trophic position	3.82[1]	4.02[1]	4.06[1]	4.36[1]	4.54[1]
	Carbon range	-3.76	-2.09	-4.00	-1.36	-3.53
C. carpio	Min. trophic position	2.30	2.98	2.51	3.08	3.23
	Max. trophic position	3.03	3.44	3.35	3.96	3.94
	Carbon range	-4.16	-2.00	-4.00	-3.38	-1.08

[1] Max. trophic position denotes Food Chain Length (FCL) estimate for each river.

Table 3. Three most parsimonious models explaining variation in δ13C and trophic position (δ15N corrected baseline) of Murray cod (*M. peelii)*, golden perch (*M. ambigua*) and common carp (*C. carpio*) sampled in LTIM selected areas (River).

Model number	Response variable	Model	DF	AIC	Pseudo R^2
1	δ13C	~factor(Species)+loglength:factor(Species)+factor(River)	2	607	0.64
2	δ13C	~loglength+factor(Species)+factor(River)	2	626	0.63
3	δ13C	~loglength+loglength:factor(Species)+factor(River)	2	637	0.62
4	Trophic position	~ factor(Species)+loglength:factor(Species)+factor(River)	2	179	0.86
5	Trophic position	~loglength+factor(Species)+factor(River)	2	188	0.86
6	Trophic position	~loglength:factor(Species)+factor(River)	1	193	0.86

Table 4. Most parsimonious model explaining variation in trophic position (δ15N corrected baseline) of Murray cod (M. peelii), golden perch (M. ambigua) and common carp (C. carpio) sampled in LTIM selected areas (River). See Table 3, Model 4.

Variable		value	SE	p-value
Species	*M. peelii*	0.35	3.80	NS
	M. ambigua	-0.77	3.96	NS
	C. carpio	1.57	3.88	NS
River	Edward-Wakool	0.63	3.82	NS
	Goulburn	0.57	0.08	P < 0.0001
	Lachlan	0.04	0.09	NS
	Lower Murray	0.59	0.09	P < 0.0001
	Murrumbidgee	1.03	0.08	P < 0.0001
Length:Species	*M. peelii*	0.95	0.15	P < 0.0001
	M. ambigua	1.74	0.48	P < 0.0001
	C. carpio	0.51	0.25	P < 0.001

Table 5. Most parsimonious model explaining variation in δ13C of Murray cod (M. peelii), golden perch (M. ambigua) and common carp (C. carpio) sampled in LTIM selected areas (River). See Table 3, Model 1.

Variable		value	SE	p-value
Species	*M. peelii*	-13.38	13.44	NS
	M. ambigua	-11.98	13.97	NS
	C. carpio	-19.8	13.48	NS
River	Edward-Wakool	1.01	0.33	P < 0.001
	Goulburn	1.07	0.31	P < 0.0001
	Lachlan	3.58	0.31	P < 0.0001
	Lower Murray	3.96	0.32	P < 0.0001
	Murrumbidgee	2.24	0.29	P < 0.0001
Length:Species	*M. peelii*	1.06	0.54	0.052
	M. ambigua	0.46	1.69	NS
	C. carpio	3.16	0.9	P < 0.001

Table 6. Dissolved organic carbon (DOC) and Chlorophyll a (Chlor a) concentrations in Murray-Darling Basin rivers sampled for stable isotope analyses.

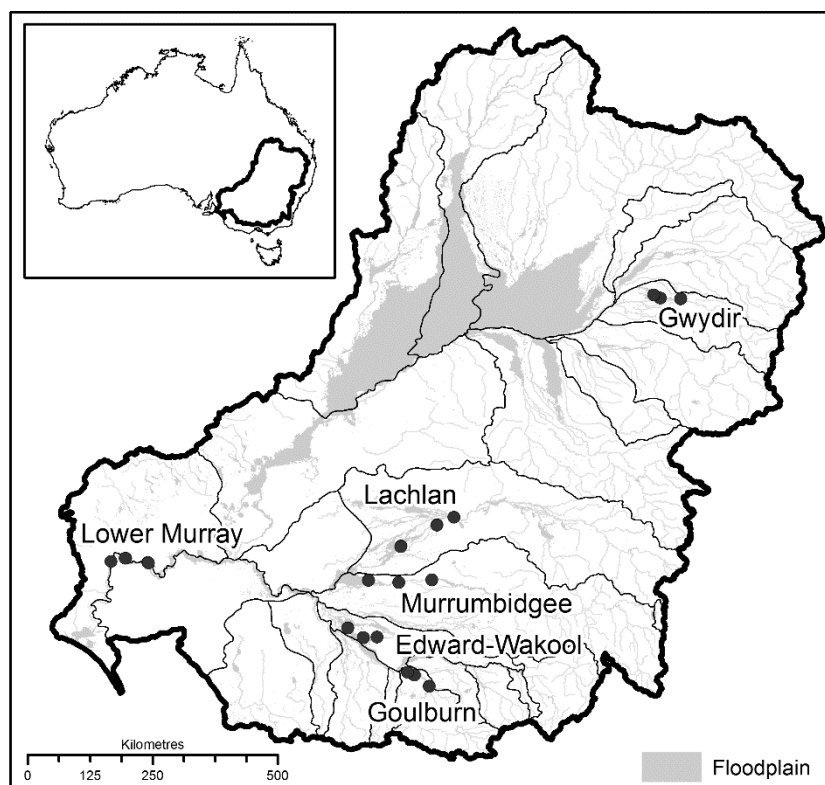| River	DOC (mgC/L)			Chlor *a* (ug/L)		
	n	mean	SE	n	mean	SE
Edward-Wakool	5	3.95	± 0.24	6	17.65	± 1.98
Goulburn	6	2.90	± 0.04	6	13.10	± 3.17
Gwydir	6	11.20	± 0.19	6	12.33	± 2.50
Lachlan	6	10.45	± 0.17	6	46.48	± 5.73
Lower Murray	6	5.64	± 0.05	6	18.94	± 1.40
Murrumbidgee	6	3.53	± 0.20	6	23.46	± 7.98

Figure 1. Murray-Darling Basin rivers sampled for fish tissues and basal resources to examine trophic niche using stable isotopes (δ13C and δ15N) in 2018. Black points illustrate the locations of two basal isotope sampling sites in the upper, middle and lower end of each reach. Fish tissues were not available for stable isotope analyses in the Gwydir.
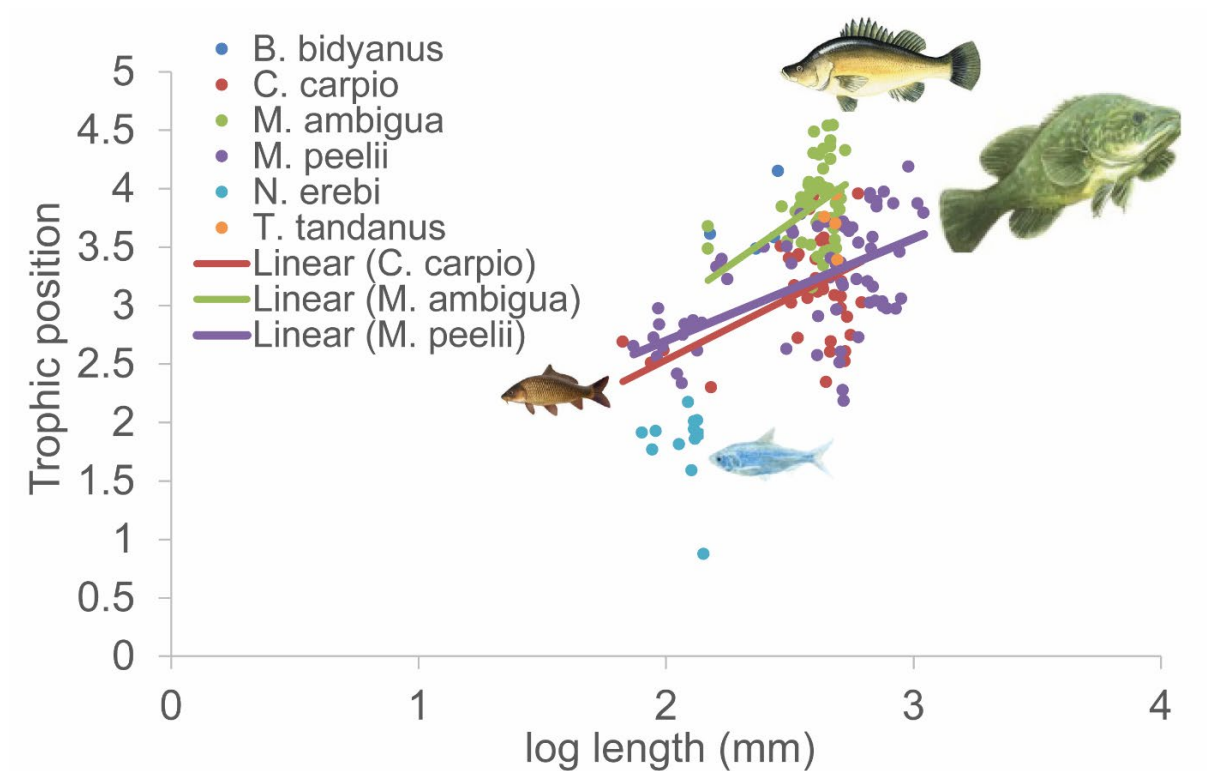
Figure 2. Trophic position (δ15N corrected baseline) of Murray cod (*M. peelii*), golden perch (*M. ambigua*) and common carp (*C. carpio*) varied significantly among species and increased with length (mm). Silver perch (*B. bidyanus*),

Eel-tailed catfish (*T. tandanus*) and Bony herring (*N. erebi*) are shown for illustrative purposes only. Note, length is reported on a $\log_{10}$ scale (e.g. 3 = 1000 mm; 2 = 100 mm).
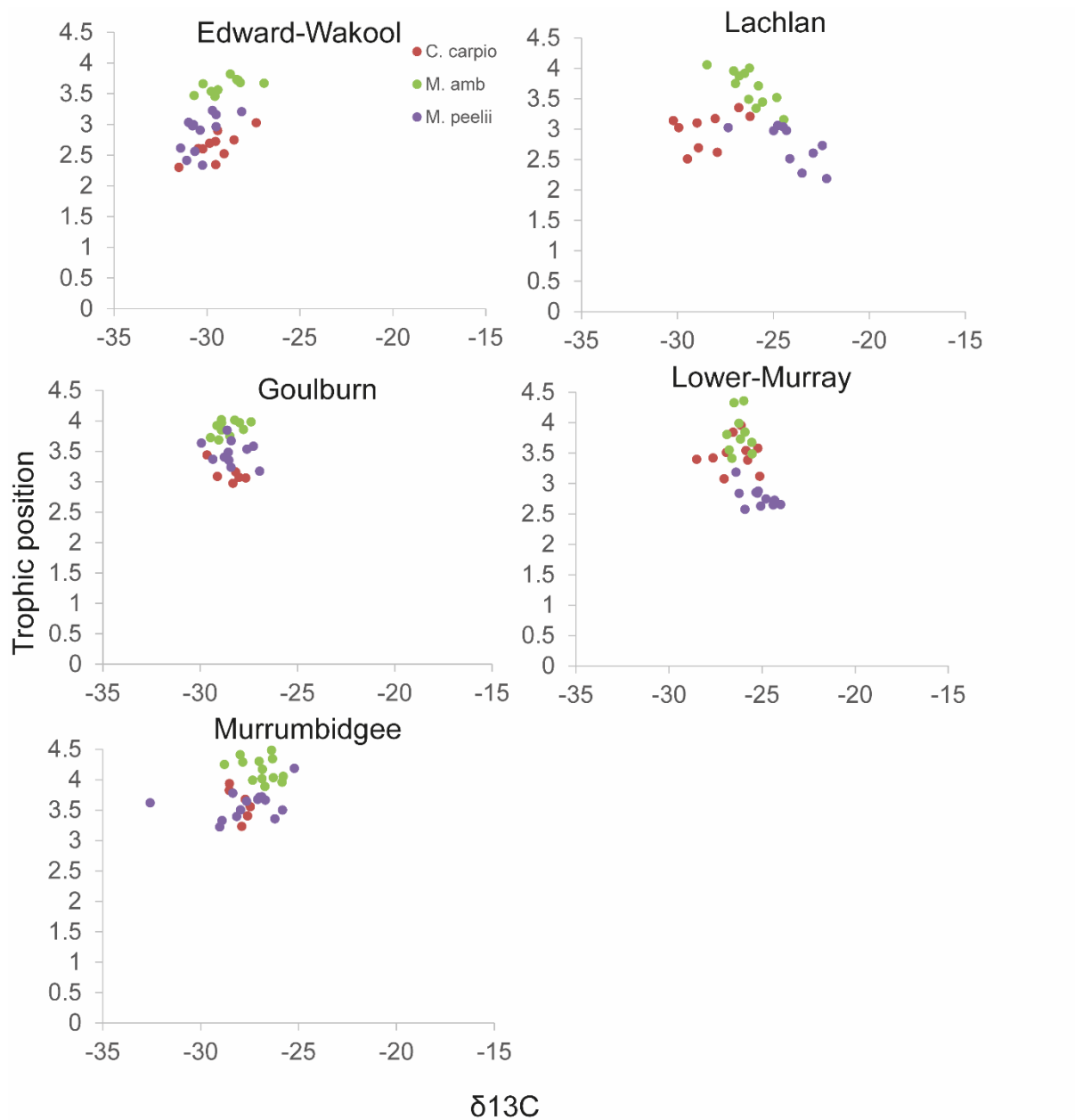

Figure 3. Trophic niche (δ15N corrected baseline and δ13C) of Murray cod (*M. peelii*), golden perch (*M. ambigua*) and common carp (*C. carpio*) varied significantly among species and LTIM selected areas (Rivers).
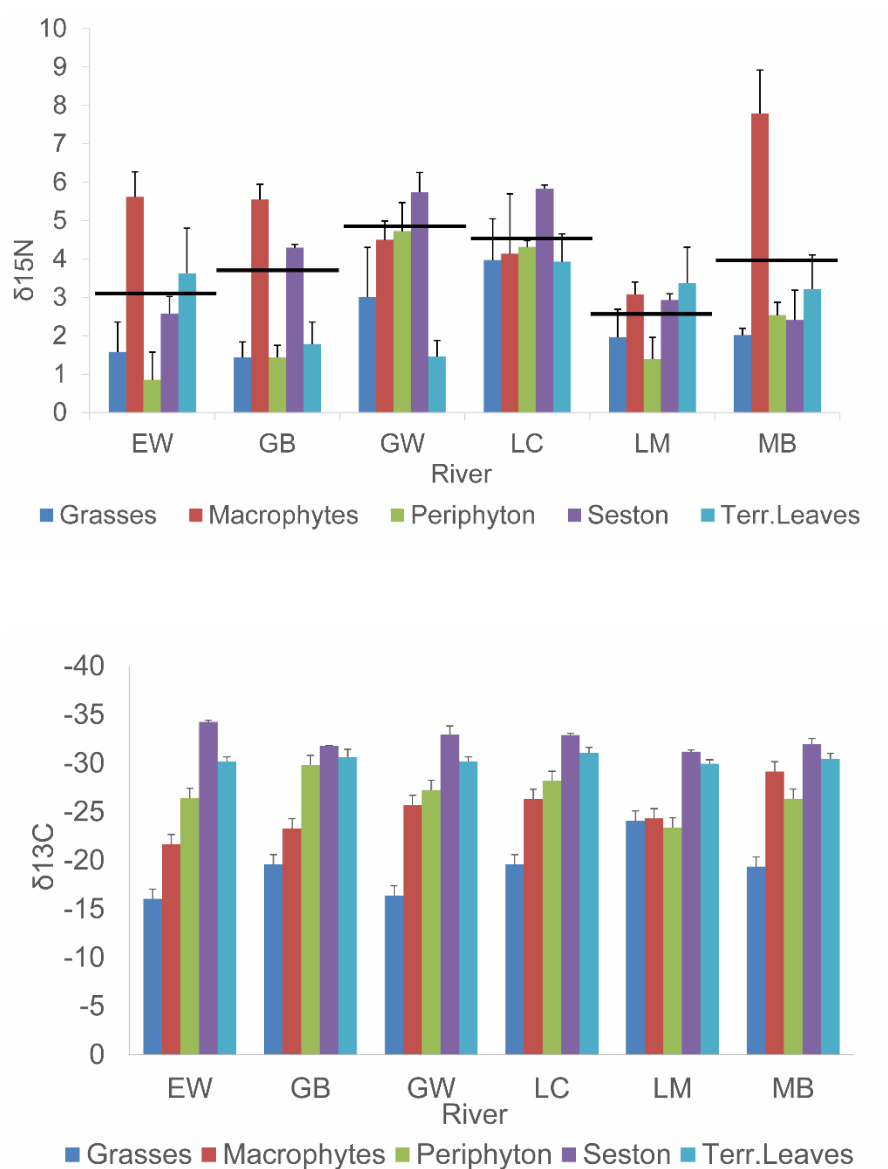
Figure 4. Variation in δ15N (top panel) and δ13C (bottom panel) stable isotope means (± SE) for LTIM selected areas (Rivers) and basal resource types. Rivers=Edward Wakool (EW); Goulburn (GB); Gwydir (GW); Lachlan (LC); Lower Murray (LM); Murrumbidgee (MB). Black horizontal lines in the top panel illustrate the δ15N baseline used to correct estimates of fish trophic position in each selected area.

Appendix 1: Research Activity Reports

Appendix 1.5 Modelling energy flow from environmental water

Modelling inundation and bioenergetics within the Gwydir Wetlands

Full manuscript in preparation:

Galen Holt, Ashley Macqueen, Ross Thompson, Darren Ryder, Nick Bond, Barbara Robson, Paul McInerney, Ben Gawne, Darren Baldwin, Rebecca Lester (in prep) Modelling bioenergetics within identified production sites.

Research Question

Food webs are one of several critical ecosystem functions that are important in sustaining patterns of diversity in the Murray-Darling Basin (MDB). A food web describes the pathways that energy and essential nutrients take through an ecosystem, from primary producers (e.g. biofilm, detritus, green algae and macrophytes), through consumers (e.g. zooplankton), to apex predators such as fish and waterbirds. Improved understanding of the influence of environmental flows on food webs is likely to complement our understanding of the influence of flow on habitat and connectivity and, in combination, this knowledge will enable better management of environmental flows within the Basin. But, while most major conceptual models of river ecosystems propose food webs as a critical ecosystem function, our knowledge of the influence of flow on food webs is not robust enough to make specific predictions about how flow influences outcomes such as the production of larval fish biomass.

Our primary goal was to link individual management decisions about environmental watering to potential ecological outcomes by developing a model of energy pathways from water provision through to larval fish. We sought to integrate knowledge arising from the EWKR project as a whole, LTIM data and local area knowledge, while explicitly accounting for uncertainty in that knowledge and identifying knowledge gaps.

Using our model, we pursued two interrelated lines of investigation:

1) Ranking environmental watering scenarios in terms of their trophic-dependent fish outcomes; and

2) Identifying and quantifying the main knowledge gaps within the model that may affect our ability to rank management scenarios based on fish outcomes.

Research Outcomes Summary

We developed a simulation-based quantitative food web model that uses characteristics of environmental watering events as inputs and ranks those watering events based on simulated larval fish biomass, using the Gwydir wetlands as a test case. The model was developed as a series of compartmentalized sub-models, including inundation, production, carbon transfer through the food web, fish production and scenario comparison (illustrated conceptually in Figure 1). Each of these sub-models was based on available data, particularly those collected by EWKR and LTIM (Figure 1).
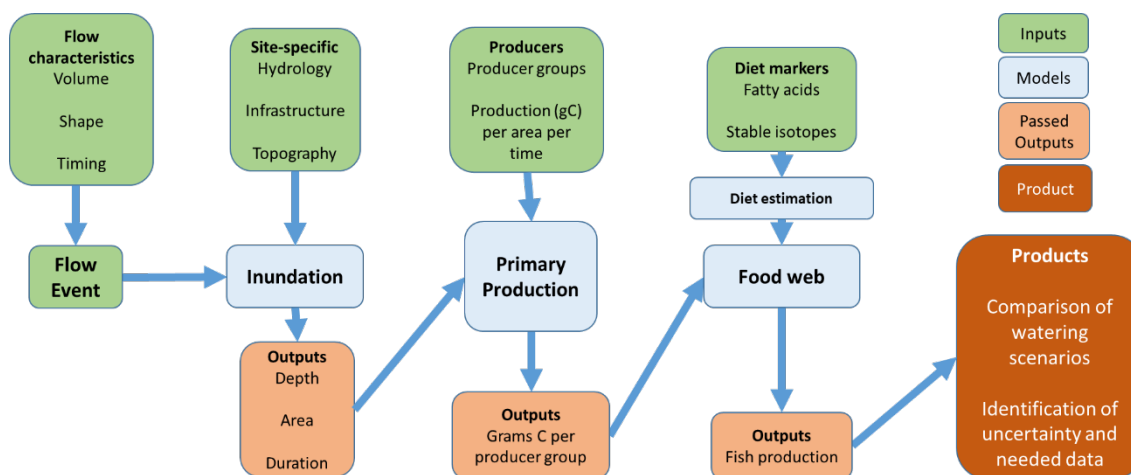
Figure 1. Structure of the food web model developed including inputs (green), sub-models (blue), outputs (beige) and the final product: a ranking of individual watering events and assessment of uncertainty associated with the inputs and outputs.

We used our model to compare a set of watering scenarios, guided by local water managers for the Gwydir, to provide information and illustrate the sorts of questions the model can address. Using a current environmental watering strategy as a baseline, we varied the rate of flow delivery, the duration of the flow and the proportion of flow delivered to the two target catchments (Gwydir and Gingham). For a given amount of environmental water, increasing the inundation duration yielded better fish outcomes than increasing the daily flow rate. Increases in the total amount of environmental water provided tend to have diminishing returns, likely due to the shape of the floodplain, tending to explain why longer durations were more effective in producing fish biomass than higher daily flow rates. Similarly, topographical differences between catchments yielded different fish biomasses depending on the proportion of water delivered to each catchment.

Our model yielded several key, general findings related to how primary production or diet differed among scenarios (Figures 2 and 3):

1) Fish biomass was proportional to the area and duration of inundation if diet quality and composition did not change among scenarios (Figure 3 between panels);

2) More flow, whether from increasing the daily flow or the duration of e-flow events yielded more area and duration of inundation, and therefore more fish (assuming quality and composition are constant) (Figure 3, x-axis and linetype), but there were differences among catchments (seen in the increase of inundation and fish biomass with increasing allocation to Gingham catchment, Figure 3);

3) For any given inundation area and duration, fish biomass could vary widely, based on uncertainty associated with production rates and diet composition (with example differences shown in Figure 2b by the size of the violins for each scenario).

4) Absolute fish biomass produced by a given scenario was more variable than the overall scenario rankings. This uncertainty was driven by shifts in the mix of producers (diet quality) or fish diet (composition) among scenarios (illustrated in the extreme in Figure 2b).
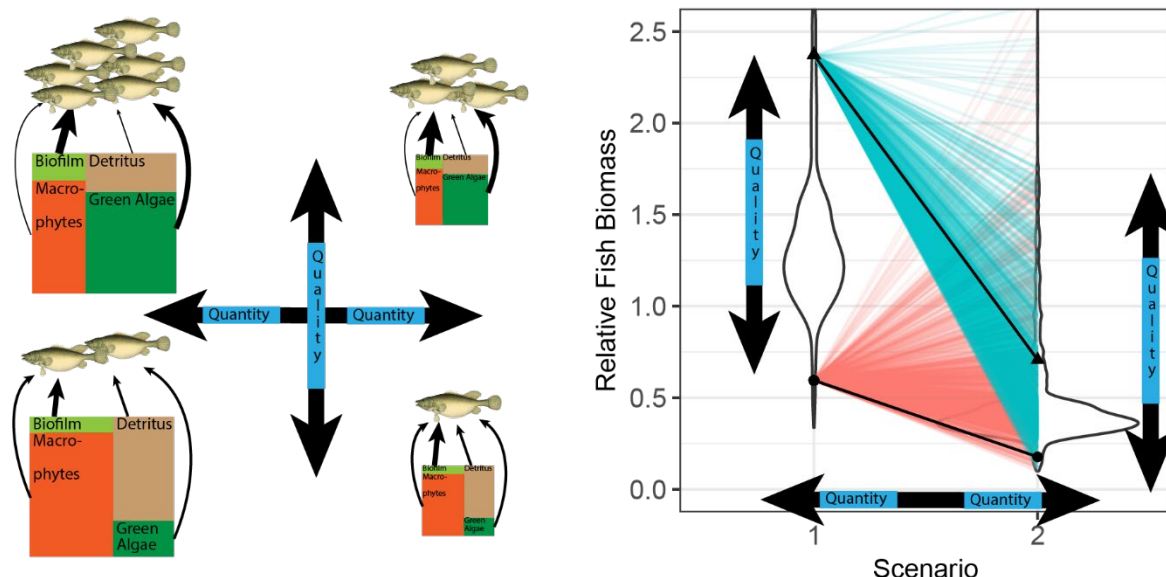
Figure 2. Potential variability around each of two scenarios is represented by violin plots, with widths indicating the number of different producer mixes and diet compositions that yield a given fish biomass. If inundation varies between scenarios, there is an overall shift in the quantity of production, illustrated in the left panel as a change in size of the producer boxes, and seen in the right panel as an average difference in the height of the violins. Within any individual scenario, however, variation in fish biomass estimates vary primarily due to the quality of production. Production quality is the combined effect of mix of producers and their energetic pathways to fish (represented by the schematic diagrams on the left-hand panel, illustrating the relative proportions of each producer type), both selected from a probabilistic distribution of available data. The highest quality is represented by the top of each violin plot, with the lowest diet quality at the bottom. The black lines illustrate the situation where diet quality is consistent between scenarios for two different reference diet qualities. Because diet quality is unchanged, shifts in the amount of production among scenarios is proportional to the area inundated. Here there is little uncertainty around the ranking of scenarios even though there is uncertainty in final fish biomass. In contrast, the coloured lines illustrate the situation where quality changes among scenarios (as it might if different areas were inundated under different scenarios). In this case, the specific shifts in quality determine the final scenario ranking (illustrated by lines connecting all possible outcomes). These shifts may accentuate or counter the effect of changes in the quantity of production and the relative ranking of the scenarios can change. A key question is what factors yield high quality production (e.g. high levels of green algae), and whether managers can target watering to produce disproportionately high fish biomass with any individual environmental watering event.
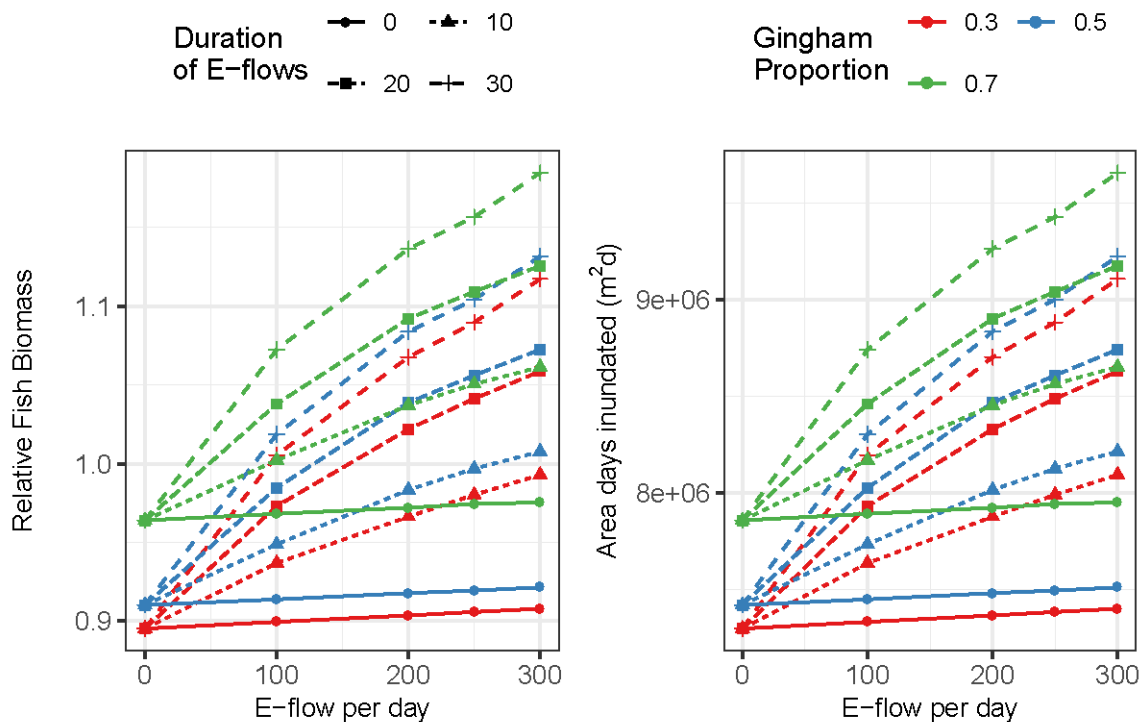
Figure 3. Increasing environmental water by either the amount per day or the number of days increases inundation, and has a corresponding exactly proportional increase in fish biomass provided diet quality and composition are unchanged. The catchment to which water is delivered interacts with these terms, and in this illustration, increasing the proportion of flow allocated to Gingham increases the inundation and fish provided by a given flow.

Ecological data (the inputs to our model) are always uncertain and these uncertainties influence the outputs of each sub-model and then propagate to affect the distribution of possible fish outcomes from a given watering scenario. Exploring this uncertainty identified two critical knowledge gaps which, if filled, would provide greatest benefit for comparing management actions.

1) The structure of the food web itself, which determines diet composition (Figure 2), and whether that food web changes with the type of environmental watering.

   - For example, does the dominant source of carbon for fish change if production shifts from being dominated by green algae to being dominated by macrophytes?

2) Rates of production by primary producer groups (Figure 2), and specifically how they might change in different areas or among different watering scenarios.

   - For example, if low open herblands are inundated, do you get a different mix of producers (analogous to diet quality) compared with when club rushes are inundated instead?

Other important knowledge gaps that influenced the outcomes included:

3) Estimates, variability and drivers of production rates of producer groups from modelled area to better estimate fish biomass and identify potential shifts among scenarios;

4) Whether fish (and intermediate consumers) shift their diets in response to different producer groups;

5) How inundation aligns with producer groups in space to yield different sets of producers under different scenarios, and therefore enabling managers to target particular areas that yield high quality mixes of producers.

Water Management Application

Our key finding was that the ranking of watering scenarios depended on whether the diet quality or composition changed with environmental watering scenario or not (Figure 2). If neither change, the ranking of scenarios is determined by the area and duration of inundation and scenarios differ only in the quantity of production. In this case, the result is a relatively homogeneous landscape where more water equates to more production and that leads to more fish. So, while the actual amount of production may vary, the relative ranking does not (see black arrows in Figure 2b). In contrast, if either the diet quality, composition or both changed, depending on the watering scenario, then the relative ranking of scenarios would also change, suggesting a more heterogeneous landscape. This would mean that a scenario that might result in more fish biomass than a second scenario under some diet compositions (for example) could result in disproportionately more or less fish biomass than that same second scenario under other diet compositions (see the range of possible outcomes represented by the overlapping orange and blue lines in Figure 2b, suggesting that in some circumstances, one scenario produces more fish biomass and in other circumstances, a difference scenario produces more). Therefore, there would be no clear ranking of scenarios from most favourable to least favourable without additional information about how those scenarios affect diet composition and quality (i.e. the mix of producers represented by the different size of boxes in Figure 2a).

Thus, untangling whether the diet quality or composition change with different watering scenarios is critical to the use of this model to support management. Understanding the sources of uncertainty in the model can generate better hypotheses prior to environmental watering actions, and aid interpretation of results, particularly when unexpected events occur.

For example, by targeting shifts in diet composition and producer quality, watering events can be designed to disproportionately benefit fish. In particular, green algae have the potential for high production rates and can contribute a large proportion of the energy used by fish. While we currently have few data to target watering events to the production of green algae, it is likely that managers on the ground may have local knowledge to achieve this. These results highlighting the importance of green algae complement findings from the Ovens and Gwydir, where green algae was identified as containing the highest quality fatty acid profile and as growing the biggest fish in mesocosm experiments. Here we find that fish appear to prefer to eat green algae and that, when they do, the estimated biomass is greatest.

Appendix 2: Theme Data Inventory

Activity	Custodian	Contact
W1.1 Review of the influence of flow on lowland river food webs and the development of conceptual models and matrix of current best knowledge.	La Trobe University	Paul McInerney
W1.2 Review of food web indicators into monitoring and evaluation of environmental flows	La Trobe University	Paul McInerney
W1.3 A review of approaches to modelling predictive capacity; Monitoring and evaluating trophic dynamic responses to hydrological manipulations in riverine systems	La Trobe University	Paul McInerney
W2.1 Fish field program – Identifying the basal resources supporting fish recruitment in lowland rivers	La Trobe University	Paul McInerney
W2.2 Waterbird food requirements research program	CSIRO	Heather McGinness
W2.3 Mesocosm Experiments: Zooplankton and fish - basal resource transfer efficiency between a range of basal resources and to first order consumers	University of New England	Darren Ryder
W2.3 Mesocosm Experiments: Zooplankton and fish - basal resource transfer efficiency between a range of basal resources and to first order consumers	University of New England	Darren Ryder
W2.4 Basin scale trophic niche indicator	Charles Sturt University	Keller Kopf
Completion of the ACEAS code and manuscript submission	La Trobe University	Nick Bond
W4 Modelling bioenergetics within production sites	Deakin University	Rebecca Lester

Appendix 3: Theme Outputs

Work Component	Activity	Output	Manuscript / report citation or Factsheet title
W1 Conceptualisation/Foundation activities	W1.1 Review of the influence of flow on lowland river food webs and the development of conceptual models and matrix of current best knowledge.	Development of a summary table for consultation with managers and policy makers; at least one face-to-face workshop with managers, and an additional writing workshop with the leadership team	
W1 Conceptualisation/Foundation activities	W1.2 Review of food web indicators into monitoring and evaluation of environmental flows	Scientific manuscript	Robson BJ, Lester RE, Baldwin DS, Bond NR, Drouart R, Rolls RJ, Ryder DS, Thompson R (2017) Modelling food-web mediated effects of hydrological variability and environmental flows. Water Research. 124 108-128
W1 Conceptualisation/Foundation activities	W1.3 A review of approaches to modelling predictive capacity; Monitoring and evaluating trophic dynamic responses to hydrological manipulations in riverine systems	Scientific manuscript	Rolls, R. J., Baldwin, D. S., Bond, N., Lester, R. E., Robson, B. J., Ryder, D. S., Thompson, R. M., & Watson, G. A. A framework for evaluating food-web responses to hydrological manipulations in riverine systems. Journal of Environmental Management Volume 203, Part 1, 1 December 2017, Pages 136-150
W2 Identifying critical basal resources	W2.1 Fish field program – Identifying the basal resources supporting fish recruitment in lowland rivers	Scientific manuscript	
W2 Identifying critical basal resources	W2.2 Waterbird food requirements research program	Report, data for food web modelling	
W2 Identifying critical basal resources	W2.3 Mesocosm Experiments: Zooplankton and fish - basal resource transfer efficiency between a range of basal resources and to first order consumers	A manuscript will provide the basis for presentations to managers and development of summary material to be made available through the web page	
W2 Identifying critical basal resources	W2.3 Mesocosm Experiments: Zooplankton and fish - basal resource transfer efficiency	Empirical data that expresses relative magnitudes of energy pathways to taxa of interest that will	

Work Component	Activity	Output	Manuscript / report citation or Factsheet title
	between a range of basal resources and to first order consumers	inform future model development.	
W2 Identifying critical basal resources	W2.4 Basin-scale resource use by fish larvae - basal resource transfer efficiency between a range of basal resources and to first order consumers	A manuscript will provide the basis for presentations to managers and development of summary material to be made available through the web page	
W2 Identifying critical basal resources	W2.4 Basin-scale resource use by fish larvae - basal resource transfer efficiency between a range of basal resources and to first order consumers	Empirical data that expresses relative magnitudes of energy pathways to taxa of interest that will inform future model development.	
W3 Identifying important sites of production	W3 Identifying important sites for production	Empirical data - spatial and temporal variability; Generalised identification of types and spatial extent of productive areas	
W4 Modelling bioenergetics within production sites	Completion of the ACEAS code and manuscript submission	Code	
W4 Modelling bioenergetics within production sites	W4 Modelling bioenergetics within production sites	Scientific manuscript on ACEAS model	
W4 Modelling bioenergetics within production sites	W4 Modelling bioenergetics within production sites	Summary report of food web modelling	

Appendix 4: Theme Engagement and Communications Activities

Category	URL	Content / Activity
Social Media	https://www.facebook.com/pg/TheMDFRC/posts/	Do you want to know more about how hydrological regimes and environmental flows can influence spatial and temporal patterns of river and floodplain production in Murray–Darling Basin rivers? Are you interested in the transfer of energy in river-floodplain networks and how to incorporate these into monitoring? Then don't miss the opportunity to hear Dr Darren Ryder present on these topics at the River Symposium in Brisbane. For conference details go to http://riversymposium.com/ This research is part of the Murray-Darling Basin Environmental Water Research and Knowledge (MDB EWKR) project, a collaborative $10m Commonwealth Environmental Water Office funded research project led by the Murray-Darling Freshwater Research Centre.
Social Media	https://www.facebook.com/pg/TheMDFRC/posts/	Coming soon: The Murray-Darling Basin Environmental Water Knowledge and Research (MDB EWKR) project's fish and food web teams conducted field work over summer. These teams are looking at the movement of energy and nutrients through the food web and the nutrient profiles of various habitats in the main channel, anabranches and wetlands of the Ovens River. Highlights of the fieldwork and subsequent laboratory analyses has been filmed and is now being produced for release on social media and the MDB EWKR website. So watch this space!
Social Media	https://www.facebook.com/pg/TheMDFRC/posts/	Researchers working on the food web component of the Murray-Darling Basin Environmental Water Knowledge and Research (MDB EWKR) posted photos of a greenhouse with some empty containers and pumps in April. Since then, monocultures of green algae and cyanobacteria have been grown, as well as biofilms with diverse algal and microbial communities and dissolved carbon. This will allow a broad range of different food regimes to be established for microinvertebrates, with the aim of manipulating their growth and nutritional value. Once reared on these different quality food sources, the impact on larval fish growth will be analysed. A number of emergence experiments from channel and floodplain sediments have identified a predictable sequence of food availability and zooplankton community composition following inundation. Outcomes from these experiments will establish links between flow, food quality and successful fish recruitment that can be further explored and validated in field trials. This work is being led by Dr Darren Ryder from the University of New England, as a part of the MDB EWKR project.

Category	URL	Content / Activity
Website		In April, we posted photos of a greenhouse with some empty containers and pumps, ready to go. Since then, we have grown monocultures of green algae and cyanobacteria, as well as biofilms with diverse algal and microbial communities and dissolved carbon. These will allow us to create a broad range of different food regimes for microinvertebrates, with the aim of manipulating their growth and nutritional value. Once reared on these different quality food sources, we will explore their impact on larval fish growth. A number of emergence experiments from channel and floodplain sediments have identified a predictable sequence of food availability and zooplankton community composition following inundation. Outcomes from these experiments will establish links between flow, food quality and successful fish recruitment that we can further explore and validate in field trials. Follow us on Facebook for more project updates – find us at @The MDFRC. For more information contact: Paul McInerney (Food Web Theme Coordinator) Email: p.mcinerney@latrobe.edu.au Phone: (02) 6024 9649 Darren Ryder (Food Web Leadership Group) Email: dryder2@une.edu.au Phone: (02) 6773 5226
Website		Do you want to know more about how hydrological regimes and environmental flows can influence spatial and temporal patterns of river and floodplain production in Murray–Darling Basin rivers? Are you interested in the transfer of energy in river-floodplain networks and how to incorporate these into monitoring? Then don't miss the opportunity to hear Dr Darren Ryder present on these topics at the River Symposium in Brisbane. For conference details go to http://riversymposium.com/
Printed Article		Power Supply - Bond N, Lester R 27-28pp
Printed Article		Exploring links in the food chain Gawne B 24-26pp
Social Media	https://www.youtube.com/watch?v=TEHauiuzonc	MDFRC EWKR Food Webs
Presentation		How does hydrology affect food webs and why does it matter?
Presentation		Food web responses to hydrologic regimes in floodplain rivers.

Category	URL	Content / Activity
Social Media	https://www.facebook.com/pg/TheMDFRC/posts/	Dr Darren Ryder will be presenting at and chairing the food web session at the Australian Society for Limnology (ASL) congress in Sydney next week. He will be presenting about how hydrological regimes and environmental flows can influence spatial and temporal patterns of river and floodplain production in Murray-Darling Basin rivers. He will also be talking about frameworks for incorporating food web attributes into monitoring, and how to negotiate the myriad of options for analysing and modelling trophic dynamics in river floodplain systems. This research is part of the Murray-Darling Basin Environmental Water Research and Knowledge (MDB EWKR) project, a collaborative $10m Commonwealth Environmental Water Office funded research project led by the Murray-Darling Freshwater Research Centre. For conference details go to http://aslconference.org.au/
Social Media	https://www.facebook.com/pg/TheMDFRC/posts/	Dr Paul McInerney is gearing up to present at the Australian Society for Limnology (ASL) congress in Sydney next week. He will be talking about how environmental flows can support a boom of aquatic invertebrate biomass in floodplain wetlands, and mechanisms that drive food web productivity concordant with the flood pulse concept. Paul is the coordinator of the food web theme group for the MDB Environmental Water Knowledge and Research (EWKR) project. For conference details go to http://aslconference.org.au/
Presentation		Flooding drives a macroinvertebrate biomass boom in ephemeral floodplain wetlands
Presentation		Food web responses to hydrologic regimes in floodplain rivers.
Social Media	https://www.facebook.com/pg/TheMDFRC/posts/	EWKR paper published! Rob Rolls is the lead author in the paper: 'A framework for evaluating food-web responses to hydrological manipulations in riverine systems' in the Journal of Environmental Management. The paper provides a framework for monitoring the effects of hydrological regimes on riverine trophic dynamics. Contributing authors are Darren Baldwin, Nick Bond, Rebecca Lester, Barbara Robson, Daren Ryder, Ross Thompson and Garth Watson. Rob can be contacted at robert.rolls@canberra.edu.au for more information about the paper.
Website		Rob Rolls is the lead author in the paper: `A framework for evaluating food-web responses to hydrological manipulations in riverine systems' in the Journal of Environmental Management. The paper provides a framework for monitoring the effects of hydrological regimes on riverine trophic dynamics. Contributing authors are Darren Baldwin, Nick Bond, Rebecca Lester, Barbara Robson, Daren Ryder, Ross Thompson and Garth Watson. Rob can be contacted at robert.rolls@canberra.edu.au for more information about the paper.
Presentation		Bond N (2018) Quantifying the effects of increasing floodplain connectivity and food-web structure on ecosystem carry capacity. Measuring productivity by EWKR and LTIM with the aim to develop a monitoring strategy to inform water delivery to improve river productivity
Social Media	https://www.facebook.com/LaTrobeCFE/	
Social Media	https://www.facebook.com/LaTrobeCFE/	

Category	URL	Content / Activity
Presentation		Identifying critical basal resources for fish recruitment
Presentation		The EWKR food web team are interested in understanding which environmental flow regimes can best support the food webs necessary for successful breeding and survival of waterbirds and fish (known as 'recruitment'). To improve our understanding of MDB ecosystems, food web group members Darren Ryder, Paul McInerney and Keller Kopf have conducted their research at three different spatial scales – mesocosm, site and basin wide. To find out more about what their results mean for ewater management come along and see their presentations. Mesocosm Scale Research, Darren Ryder (University of New England) – Understanding links between e-water delivery, food quality and consumers: lessons from mesocosm experiments. Site Scale Research, Paul McInerney (La Trobe University, Centre of Freshwater Ecosystems)– Basal resource quality and energy flow in a lowland river food web. Basin Scale Research, Keller Kopf (Charles Sturt University) – Fish community trophic position indicator for monitoring environmental flows.
Presentation		Identifying critical basal resources for fish recruitment