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

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ABSTRACT

The use of freshwater for human consumption and agriculture has resulted in many wetland ecosystems being some of the most seriously impacted ecosystems 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. To date, research and monitoring has mainly focused 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 2 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, the biomarkers of diatoms, cyanobacteria and bacteria also increased and differed between wetlands with different hydrological regimes. 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.

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1. Introduction

The increased 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; Vörösmarty 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 2011a). The level of water use has proved to be unsustainable, both in meeting the human 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 2011b; 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 2011a; Docker and Robinson 2014). However, research and monitoring are required to assess both how water should be delivered to highly valued ecosystems and animals and the processes underlying any potential benefits.

Nutrition is the supply of materials (food) required by cells and therefore organisms and cells to stay alive. The nutritional quality 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 is 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). Elemental stoichiometry (ES), particularly the ratio of carbon to nitrogen (C:N) can also used to assess food quality, the greater the C:N the lower the quality food (Cruz-Rivera and Hay 2000; Sullivan et al. 2014). 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) and fatty acid profiling (FAP). Fatty acids (FA) are a group of compounds that comprise the majority of lipids found in all organisms. FA profiles can be used to characterise 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, FAP also has the advantage of integrating diet over weeks to months but can only be used to characterise resources consumed by freshwater biota when sources have distinct FA compositions (Traugott et al. 2013). FAP is 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 synthesised *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. Its conservation status is considered vulnerable by Australian authorities (Australian Society for Fish Biology 2016; Department of Agriculture, Water and the Environment 2018). 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 relationships between environmental water, basal resources and trophic pathways to support Murray cod populations is unknown. Murray cod diet 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 and 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.

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 for delivery 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 foodwebs 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). Cyanobacteria, phytoplankton, green algae and biofilm availability can be altered both directly, and indirectly via nutrient inputs, during environmental flows (Sheldon and Walker 1997; Ryder et al. 2006; Williamson et al. 2018). 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).

In this study our objectives were to (1) identify the importance of different basal food sources to the growth of Murray cod and if this differed between wetlands with different hydrological regimes. (2) If any differences in basal food sources would affect the composition and nutritional value of prey items and therefore the growth of Murray cod.

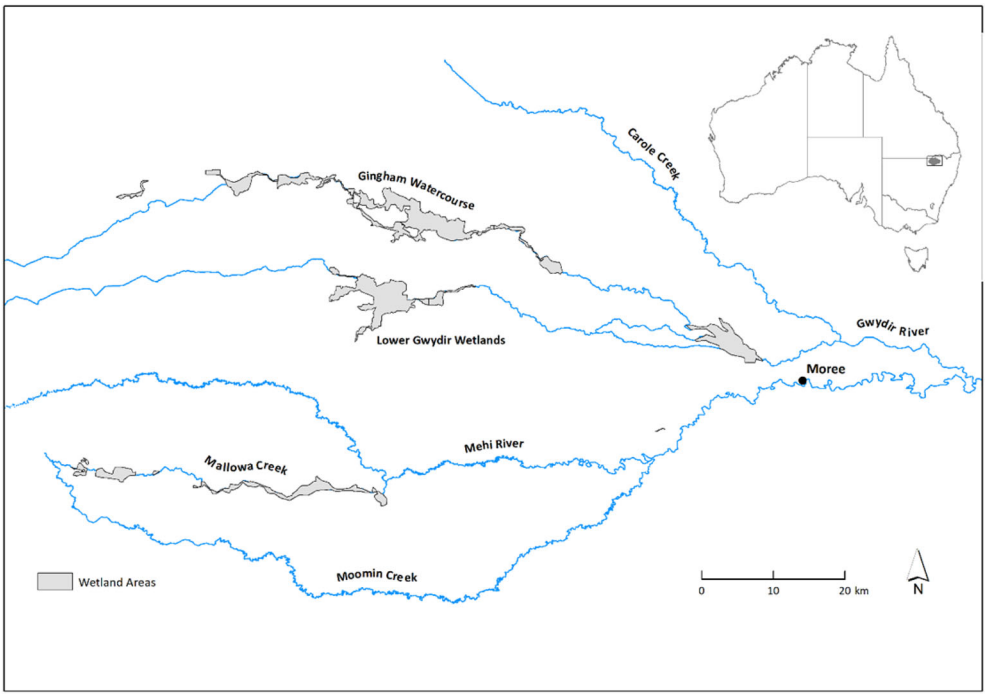

Figure 1. Location of study area.

2. Methods

2.1. Study wetlands

The Gwydir wetlands are located in the Gwydir catchment, part of the Murray-Darling Basin in northwest NSW, Australia (Figure 1). 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.

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 recorded as being 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). Since the stabilisation of the Raft, a natural accumulation of felled timber and sediments which effectively dams the river approximately 20 km 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).

2.2. 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 colonising 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 (Notostraca: *Triops australiensis*) were removed from the containers when observed, following Boulton and Lloyd (1992) and Growns et al. (2020), because either their activity within the mesocosms or their feeding behaviour reduced the populations of other invertebrates.

2.3. 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 point 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 × 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 tissue analyses 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 these 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; 400–250 µm), fine particulate organic matter (FPOM; 250–125 µm) and very fine particulate organic matter (VFPOM; 125–63 µm).

Captured larvae were anesthetised, measured for standard length, weighed and their entire alimentary canal removed and preserved in ethanol for GCA. 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).

2.4. 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.

2.5. Tissue analyses

C and N content were analysed for fish, zooplankton and the three organic sediment fractions using continuous flow isotope ratio 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 dichloromethane: methanol (CH_2Cl_2:CH_3OH) 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 utilised 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).

2.6. 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.

2.7. 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 have 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).

3. Results

3.1. 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 2). The total abundance of

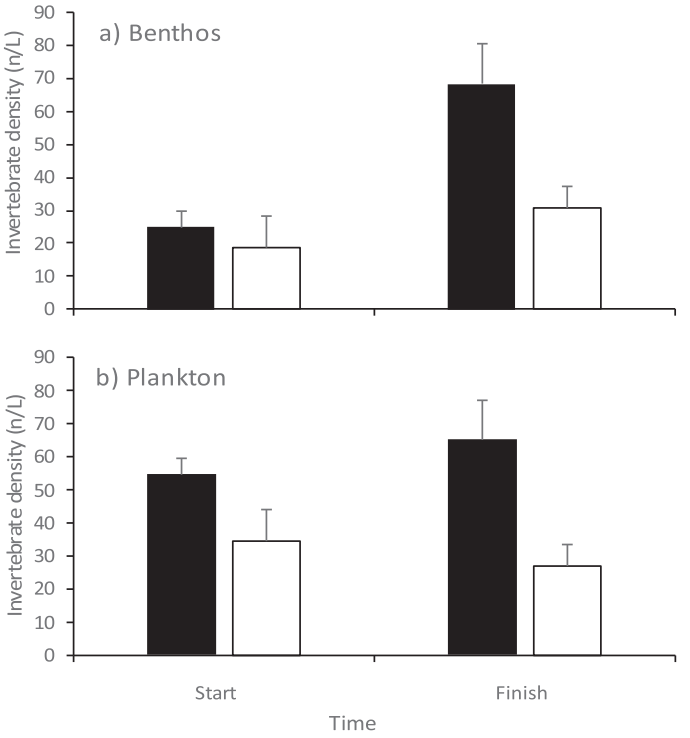

Figure 2. Mean invertebrate densities (± 1 S.E.) in (a) benthos and (b) plankton. Black columns denote Bunnor mesocosms and white Old Dromana.

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 2).

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 3a). The distance between the benthic samples and the other samples increases at the end of the experiment (Figure 3b).

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

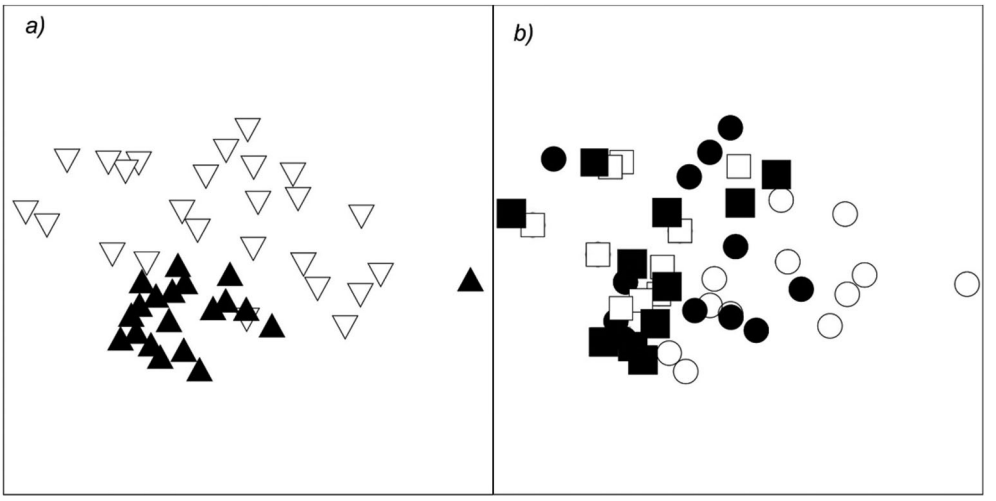

Figure 3. MDS ordination of invertebrate assemblages. (a) Coded for wetland type (black triangles = Bunnor samples; white triangles = OD samples. (b) The same ordination coded for different habitats at the start and end of the experiment (circles = benthos; squares = plankton; black shapes = start; white shapes = end).

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	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

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.

3.2. 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

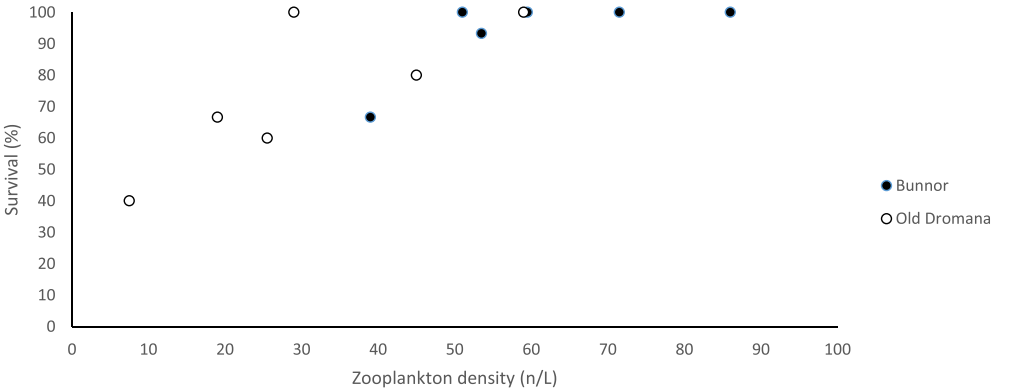

Figure 4. Relationship between zooplankton density and Murray cod larval survival. Black circles Bunnor wetland mesocosms and white circles Old Dromana mesocosms.

either mean weight or length between the wetlands, however, there was significant differences in both mean weight and length among the OD mesocosms ($F_{1,10} = 11.0$, $p = 0.0001$).

Survival of larvae differed between wetland mesocosms ($F_{1,8} = 5.6$, $p = 0.043$) with mean survival greater in Bunnor (Figure 4). 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.

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 5). In contrast, Macrothricidae/Daphniidae cladocerans in both habitats of the OD fish and Ostracoda in both wetlands and habitats appeared to be avoided.

3.3. 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.

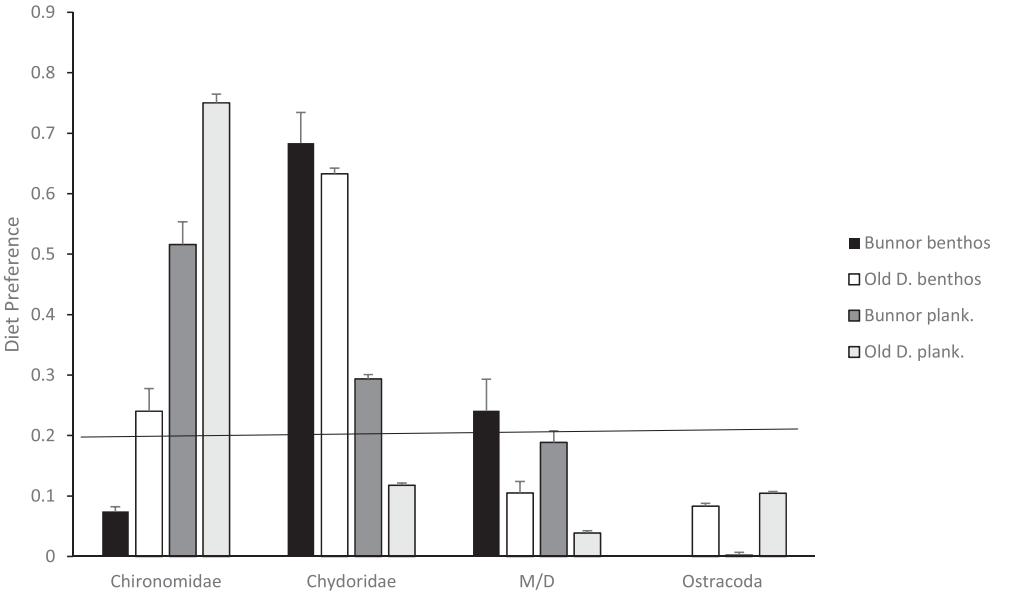

Figure 5. Manly-Chesson prey selection index α, the horizontal line is the neutral value for selectiveness.

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.

3.4. 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 6). 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.

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 7). 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.

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

		Bunnor					Old Dromana				
Fatty acid	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
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

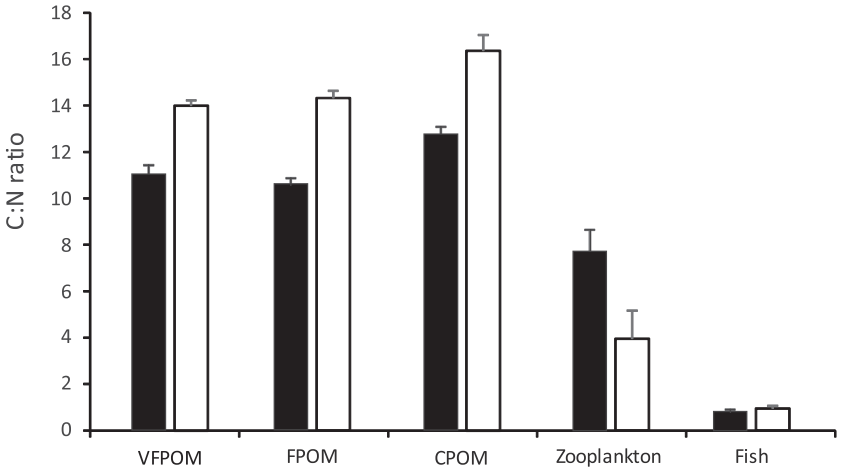

Figure 6. Mean (± 1 S.E.) carbon to nitrogen ratios in various potential food sources. Black columns are from Bunnor mesocosms and white from Old Dromana mesocosms.

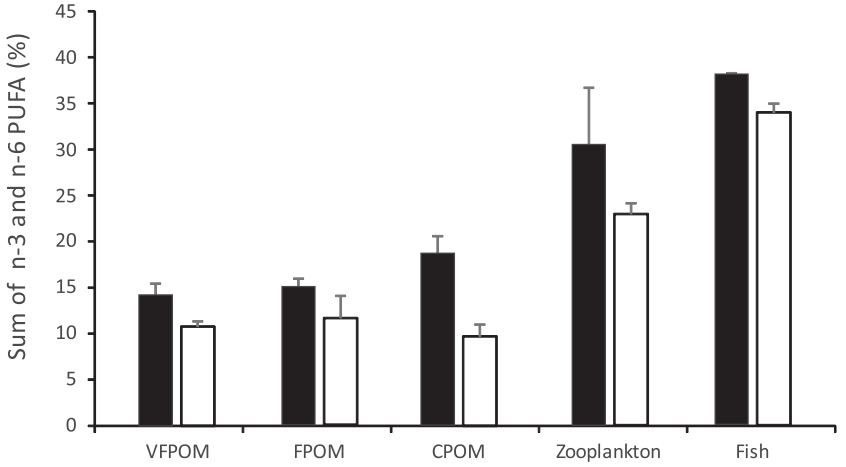

Figure 7. Mean (± 1 S.E.) sum of n-3 and n-6 polyunsaturated fatty acids (PUFA) in various potential food sources. Black columns are from Bunnor mesocosms and white from Old Dromana mesocosms.

3.5. 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 8).

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 8).

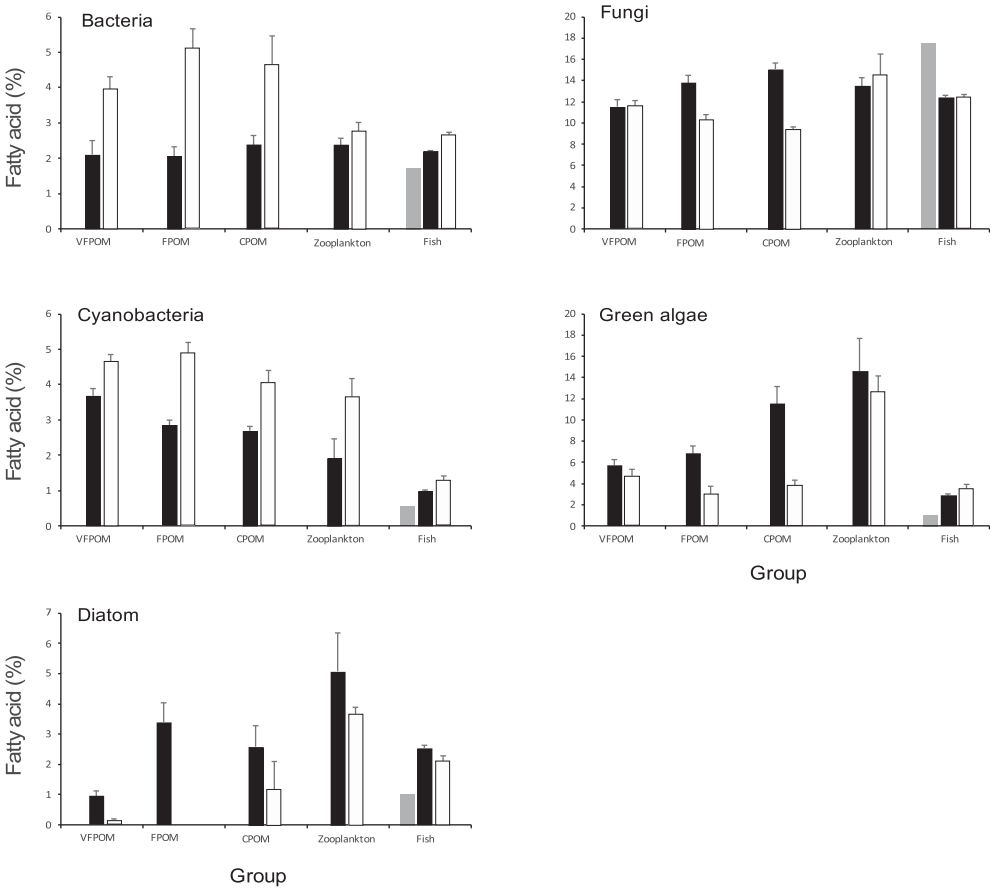

Figure 8. Mean percentage (± 1 S.E.) of fatty acid biomarkers in different substratum sizes and animal groups from Bunnor mesocosms (black columns) and Old Dromana mesocosms (white columns). Grey columns indicate the fatty acid biomarkers present in Murray cod larvae at the start of the experiment.

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 8). 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 8).

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 the percentages for VFPOM, zooplankton or fish between wetlands (Figure 8).

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 8). The greatest percentage increase was for green algae (308%) followed by diatoms (230%), cyanobacteria (204%) and bacteria (140%). However, the increases were greater for bacteria, cyanobacteria and green algae in the OD wetland.

4. Discussion

We demonstrated that the invertebrate assemblages differed between the two wetland sediments with contrasting hydrological regimes. 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 (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. 2017). *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. The food value of invertebrates to higher consumers would differ between wetlands as the percentage of PUFAs was significantly greater in the Bunnor mesocosms, however, the C:N ratios were not significantly different between the mesocosms.

The survival of Murray cod larvae was greater in the wetland with prey of greater nutritional value (based on PUFAs), 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 250–500 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. In addition, there were differences in the increases between wetlands, suggesting that the hydrological regime of a wetland influences those sources. 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 (Volkman et al. 1989; Taipale et al. 2012; Freese and Martin-Creuzburg 2013; Guo et al. 2016).

Our use of FA biomarkers to identify basal food sources is usual in the food web literature (Johns et al. 1979; Goedkoop et al. 2000; Falk-Petersen et al. 2002). However, there are some individual FA that have been ascribed to different biotic groups by different authors. For example, the FA 16:0 has been used as a biomarker separately for green algae, cyanobacteria and fungi (Vestal and White 1989; Kelly and Scheibling 2012). Biomarkers for specific species of algae are easily overlapped, such as the 18:3n6 has been simultaneously used as a marker for cyanobacteria and green algae (Meziane and Tsuchiya 2000; Hayakawa et al. 2002; de Kluijver et al. 2012; Xu et al. 2014). In addition, an individual biomarker for a specific group may not be present for environmental reasons. The FA 20:5n3 although an often consistent biomarker for diatoms is often not found in large quantities relative to other FA (Napolitano 1999) and may be found in lower light or nutrient concentrations (Ahlgren et al. 1992). Given biomarkers for individual species of plankton, aquatic and terrestrial materials are not always specific enough to identify a single source and the fact that the internal biosynthetic capabilities of most organisms have not been elucidated, some authors caution their use in food web studies (Wakeham 1995; Dalsgaard et al. 2003).

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 but that differed between the wetlands. 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.

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