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ORIGINAL ARTICLE

Dietary fatty acid profiles shape crayfish biosynthesis and performance: Implications for riverine food webs

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Abstract

1. Alteration of riverine flows can modify the structure and function of ecosystems, changing energy pathways and patterns of micronutrient transfer between trophic levels. Fatty acids (FAs) commonly are used to evaluate food quality, since some FAs required for somatic growth and physiological functions in animals must be obtained from their diet. FAs also are used in food-web studies as biotracers as a consequence of their constrained metabolic biosynthesis by animals. However, their utility may be confounded by selective retention or modification of dietary FAs by consumers.
2. We conducted a 70-day feeding trial to compare growth and survival of an abundant and widespread mesoconsumer (*Cherax destructor*, the common yabby or crayfish) fed three contrasting diets: a poor-quality detritus-based diet; a high protein invertebrate diet; and a high-quality commercial aquaculture pellet. Fatty acid profiles were obtained for each dietary treatment and contrasted with crayfish FA profiles at the end of the experiment to examine patterns of FA retention and integration. We also collected wild crayfish from floodplain wetland and river habitats, and obtained FA profiles from their stomach contents and body tissue to compare with experimental crayfish.
3. Experimental crayfish fed high-quality commercial pellets doubled in mass during the 70-day assay, invertebrate fed crayfish growth was intermediate, and growth of crayfish fed detritus was negligible. Fatty acid profiles of crayfish fed our three contrasting diets differed significantly at the end of the experiment. Proportions of the polyunsaturated omega-6 FA linoleic acid (LIN, 18:2ω6) in crayfish followed the same inequality observed in growth and diets: pellets > invertebrates > detritus. Pellet-fed crayfish preferentially assimilated greater proportions of FAs 20:4ω6 (ARA), 20:5ω3 (EPA) 18:1ω9 (OA) and 16:1ω7 (POA) into their tissue. Fatty acid profiles of floodplain crayfish differed to profiles of riverine crayfish, and floodplain crayfish had higher proportions of essential FAs ARA and LIN in their tissues.

4. Fatty acid biosynthesis by crayfish was best described by a hypothesis of FA allostasis rather than homeostasis; in this, FA profiles of crayfish were shaped by their diet, and selective integration and modification of high-quality FAs from basal resources rich in these micronutrients led to higher proportions in crayfish tissues. Here we present evidence for the conversion of shorter-chain essential FAs by freshwater crayfish to compensate for a lack of long-chain FAs in their diet.
5. We provide a necessary step for improving our understanding of micronutrient dynamics and the transfer of essential molecules between trophic levels in lowland river food webs. Floodplain habitats are known to provide higher-quality basal food resources for mesoconsumers than riverine habitats, and here we identify one mechanism by which that may be extended to subsequent trophic levels.

KEYWORDS
basal resources, *Cherax destructor*, flood pulse concept, floodplain, food quality, lowland rivers, trophic modification, yabby

1 | INTRODUCTION

Modification of natural flow dynamics in rivers can transform riverine food webs, changing the basal resources and trophic pathways driving consumer production (Kopf et al., 2019; Rees et al., 2020). There is a need to better understand how flow regimes transform food-web structure, but hindering that understanding is the challenge of resolving consumer–resource linkages. Characterising the diet of many aquatic consumers is problematic owing to the difficulty of identifying diverse, partially-digested taxa, and differential digestion rates among prey can lead to biased inferences about the relative importance of different prey to a consumer (e.g., Amundsen & Sánchez-Hernández, 2019). Modification of rivers also can transform the nutritional composition of resources at the base of freshwater food webs by altering the composition of organic material, associated microorganisms, and primary consumers (e.g., Atkinson et al., 2009; Dwyer et al., 2018; Growns et al., 2020). However, the consequences of nutritional transformations to consumer performance remain poorly understood (e.g., Dwyer et al., 2020; Ruess & Müller-Navarra, 2019). Fatty acids (FAs) can be applied to aquatic food-web studies (a) to better understand the effects of food-web transformation on consumer performance, and (b) as biomarkers, to help trace trophic pathways.

Analysis of FAs may improve our ability to predict how food-web transformation affects consumer performance (Guo et al., 2021). Growth and survival of animals can be limited by the availability of some FAs which can serve as sources of cellular energy (Jardine et al., 2020; Twining et al., 2016) and the proportions of FAs vary among basal resources and different habitats (McInerney et al., 2020). Long-chain polyunsaturated FAs (LC-PUFAs, a subset of PUFAs with ≥20 C in their acyl chains; e.g., Brett & Müller-Navarra, 1997; Guo et al., 2015; Hill et al., 2011) are especially important for maintaining a healthy physiological status and for supporting somatic growth (e.g., Kainz et al., 2010). 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 particularly important for a wide range of physiological functions and reproductive purposes (Wacker & von Elert, 2001). Since ω3 and ω6 PUFAS cannot be synthesised by most animals, they must be obtained from their diet, either as EPA, DHA and ARA, or as their precursor molecules α-linolenic acid (ALA, 18:3ω3, precursor of EPA and DHA) and linoleic acid (LIN, 18:2ω6, precursor of ARA) (Ahlgren et al., 2009). Monounsaturated FAs (MUFAs) oleic acid (OA, 18:1ω9) and palmitoleic acid (POA, 16:1ω7) have been positively associated with aquatic fungi (Funck et al., 2015) and diatoms (Taipale et al., 2013) respectively, yet are abundant in animals and are important sources of energy for fish (Tocher, 2003).

In many rivers and lakes, algae are the dominant source of energy at the base of food webs and generally are rich in LC-PUFAs (Ebm et al., 2021; Guo et al., 2016). In lowland river–floodplain ecosystems, terrestrial organic matter (litter) can be transferred to aquatic habitats in large quantities via flooding. The resulting detritus appears to provide an abundant and productive food resource for consumers (McInerney et al., 2017). Such observations are at odds with our understanding of the nutritional value of detritus, which is characterised by what is thought to be a low-quality FA profile; lack of LC-PUFAS and dominance of saturated FAs indicative of heterotrophic decomposers (e.g., 10:0, 15:0, 17:0 and their branched iso and anteiso-homologues, McInerney et al., 2020).

Use of FAs as biomarkers of trophic pathways is becoming common in aquatic food-web studies. When applying FAs as biomarkers there is an assumption that FAs are largely incorporated into consumer tissues with minimal modification (Taipale et al., 2013), such that the FA profile of the consumer matches that of its resources. However, this assumption will not hold if there is selective

retention or modification of dietary FAs by consumers, resulting in FA profiles that differ significantly from their food items (Galloway & Budge, 2020). Few studies have resolved the degree to which FA profiles of prey are modified by consumer digestion and biosynthesis.

In the present study we used laboratory feeding experiments and a field study to improve our understanding of the trophic transfer of FAs in freshwater food webs, and the consequences of changes in the FA profiles of resources to consumer performance (following the recommendation of Galloway & Budge, 2020). Our study species was the crayfish, *Cherax destructor* (Parastacidae, referred to hereafter as crayfish), a mesoconsumer that is widespread and abundant in Australia, and a critical link between basal resources and apex predators in lowland freshwater food webs (Johnston & Robson, 2009; Lawrence et al., 2002). The influences of changed hydrological regimes on the diets of crayfish and resultant implications for their populations and for taxa that rely on them for food are not well-studied and remain a significant knowledge gap.

Our first objective was to determine how the FA composition of diet affects crayfish growth and survival. We reared crayfish on three diets with significantly different FA profiles (see Appendix S1): (a) detritus, (b) invertebrates (Chironomidae) and (c) commercial crayfish pellets. These diets were selected to broadly encompass two of the major food resources that crayfish encounter in the wild (detritus and invertebrates), as well as a diet developed to be close to the optimal composition for crayfish growth and survival (commercial crayfish pellets), serving as a useful point of reference for the detritus and invertebrate diets. The percentage of LIN and OA FAs, in particular, varied strongly among diets and followed the inequality 'pellets > invertebrates > detritus'. We expected that elevated proportions of LIN and OA in diets would be responsible for high growth and low mortality of crayfish (e.g., Thompson et al., 2010; Tocher, 2003), and that the pattern of growth across these treatments would be consistent with the inequality 'pellets > invertebrates > detritus' and that mortality across treatments would be described by the opposite inequality.

Our second objective was, broadly, to determine how the FA profile of crayfish is shaped by that of their diet. We sought answers to the following questions, in an experimental setting, when fed a constant diet: (a) To what extent is the body FA profile of crayfish influenced by that of their diet? and (b) Which FAs become over- and under-represented in crayfish bodies, relative to their dietary FA profiles as a result of digestion and biosynthesis? To answer these questions, we used the same experimental setup employed for evaluating the effects of FAs on performance. Answers to these questions are required to advance the application of FAs as food-web biomarkers.

Our third objective was to determine how the FA profiles of crayfish and their diet varies among natural habitats of a river–floodplain ecosystem. We sampled crayfish from habitats in the river channel and from floodplain wetlands, and analysed the FA profiles of their body tissue and their gut contents. Modification of riverine flow regimes, and riverscapes more generally, has resulted in loss of lateral hydrological connectivity among channel and floodplain habitats, yet our understanding of how that has affected food webs is poor, partly because we have a rudimentary understanding of how consumer diets vary among river–floodplain habitats. We anticipated differences in the FA profiles of crayfish and their diets between channel and wetland habitats. Consistent with the Flood Pulse Concept (Junk et al., 1989) and other observations of the high productivity of consumers on floodplains (McInerney et al., 2017), we expected diets and crayfish from floodplain wetlands to contain FAs indicative of higher quality food resources than crayfish in the river channel. Results pertaining to this field study are interpreted in light of those from our experimental investigation.

2 | METHODS

2.1 | Study organism

Cherax destructor are native to freshwater ecosystems of south-eastern Australia and their diet is thought to comprise detritus, allochthonous and autochthonous plant material and animal prey (Beatty, 2006; Bunn & Boon, 1993). Crayfish contribute significantly to the diets of the Australian freshwater apex predators Murray cod (*Maccullochella peelii*; Percichthyidae), and golden perch (*Macquaria ambigua*; Percichthyidae) (Baumgartner, 2007; Ebner, 2006; Stoffels, 2013), and form the dominant prey items for wading birds, such as straw necked ibis (*Threskiornis spinicollis*; Threskiornithidae) and Australian white ibis (*Threskiornis molucca;* Threskiornithidae) (Carrick, 1959).

2.2 | Laboratory experiments

We measured the survival and growth of 120 crayfish fed three diets with contrasting FA profiles (n = 40 for each treatment): 1, a river red gum (*Eucalyptus camaldulensis*; Myrtaceae), leaf/detritus pellet; 2, an invertebrate-only diet (subfamily Chironominae; Aqua One Bloodworm, Aqua One® Kong's [Aust.] Pty Ltd); and 3, a diet of commercial freshwater crayfish pellets (Aquatopia Freshwater Crayfish Pellets; Aquatopia Australia Pty Ltd). Experimental crayfish and their invertebrate and freshwater crayfish pellet diets were sourced from commercial pet shops. Pet shop-sourced crayfish (used for recreational fishing bait) were of a single cohort and did not exceed 50 mm in total length at the beginning of the experiment. Detritus pellets were made from dry *E. camaldulensis* leaves soaked in mesocosm tanks exposed to sunlight for three weeks to mimic benthic litter under natural conditions. Twenty grams of soaked leaves were transferred to 100 ml of water, blended until semi-smooth, followed by a further 20 g of leaves and further blending. Agar then was added and the mixture boiled to dissolve the agar. The mixture was set in small trays and cut into 0.08-g cubes.

Crayfish are aggressively territorial, so to avoid competition and cannibalism crayfish were housed individually in 1-L plastic containers. All containers were connected to a recirculating, sump–filtration

system. Following a three-week acclimation period where all crayfish were fed a common diet consisting of carrots, each crayfish was fed 0.08 g of either frozen invertebrates, detritus or freshwater crayfish pellets once a week for 70 days. Preliminary experiments showed that this feeding rate exceeded crayfish weekly food consumption and, over the duration of the experiment, crayfish consumed on average 73 ± 3% of the weekly ration. On the day of feeding, crayfish were dried on paper towel for 5 s and weighed. Tanks were cleaned weekly to remove any excess food and faeces, and topped up with river water.

2.3 | Fatty acid analysis

At completion of the 70-day feeding trial, 10 crayfish from each treatment were selected randomly and placed in a clean tank for 48 hr to clear digestive tracts before whole crayfish were blended in a known volume of water and frozen. Blended crayfish were freeze-dried before determining FA profiles following methods used by Conlan et al. (2017) where lipid is extracted from dry samples soaked in dichloromethane:methanol (CH_2Cl_2:CH_3OH) and quantified gravimetrically on a four-figure balance. Fatty acids were esterified into methyl esters (Christie, 2003) which were separated and identified using an Agilent Technologies 7890A gas chromatography (GC) system equipped with a BPX70 capillary column (120 m × 0.25 mm internal diameter, 0.25 μm film thickness; SGE Analytical Science), a flame ionisation detector (FID), an Agilent Technologies 7693 auto sampler, and a splitless injection system. Each FA was identified relative to known external standards (Sigma-Aldrich, Inc. and Nu-Chek Prep Inc.), using GC ChemStation (Rev B.04.03; Agilent Technologies). Fatty acid profiles for experimental diets were determined following the same methods used for crayfish.

2.4 | Field collection

In order to compare the FA profiles of our experimental diets and crayfish tissue with wild crayfish, we collected specimens from two different habitats. Eight river and eight wetland sites were selected from the lower Ovens River floodplain, northeastern Victoria, Australia. Each river site was defined as a 200 m reach and wetlands known to permanently retain water were selected (for detailed descriptions of river and wetland habitats, see McInerney et al., 2020). Crayfish were sampled using collapsible shrimp traps baited with cow liver contained within plastic capsules to prevent consumption by crayfish. Three bait nets were placed randomly in c. 50 cm of water within each site and retrieved 24 hr after deployment. Ten individuals from each site were collected and frozen for FA analysis. Individual crayfish were dissected in the laboratory and their stomach contents removed to determine FA profiles of wild crayfish diets, before the remaining whole crayfish were prepared for FA analysis as described previously. We acknowledge our gut FA profiles of wild crayfish diet represent a single point in time. Nevertheless, we included these data in our study given that: (a) they comprise the first description of the FA diet of a wild population of a key consumer in Australia river–floodplain food webs, and (b) although being a temporal snapshot, the 2 (habitats) × 8 (sites) × 10 (individuals) cover a relatively broad amount of spatial and inter-individual variability in diet, making for a worthy comparison with our experimental study.

2.5 | Data analysis

2.5.1 | Objective 1: Crayfish growth on controlled diets

We used a generalised linear mixed-effects model (glmm) to determine how time (*day* of the experiment) and *diet* affected crayfish growth (R/LME4; Bates et al., 2015). Not all crayfish survived the 70 days of the experiment, but glmm models handle imbalanced data very well (Pinheiro & Bates, 2000). There was no significant difference in the survival of crayfish among treatments (log-rank test: $\chi^2 = 0.5$, $p = 0.8$; R/SURVIVAL [Therneau, 2020]). $Log_{10}mass$ was modelled as a function of fixed factors *day* and *diet*. We partitioned variation in growth rates among individuals by including *subject* as a random factor (random intercepts and slopes; Table 1). The *p*-values from glmm models often are unreliable and it is more informative to take the approach of fitting multiple models to the data, each of which represents a different hypothesis about the data being modelled (Zuur et al., 2009). The set of candidate models fitted to the data is presented in Table 1. An information-theoretic approach (Burnham & Anderson, 2002), dependent on the Akaike information criterion (AIC; Akaike, 1974), was used to select the most likely model from the candidate set.

2.5.2 | Objective 2: Biosynthesis of FAs by crayfish on controlled diets

For all multivariate analysis of FA profiles and univariate comparisons of individual FA or groups of FA (Objectives 2 and 3), we used PERMANOVA in PRIMER v7 (Clarke & Gorely, 2015) ($\alpha \leq 0.05$, 9,999 permutations of raw data, type III sums of squares). Euclidean similarities were calculated between arcsine-square-root-transformed percentages. This transformation reduced the influence of FAs comprising particularly high percentages in samples on our analysis of patterns among treatments. The SIMPER routine (Clarke, 1993) was used to identify which FAs contributed most to differences among treatments (both objectives 2 and 3).

In order to answer our first question under Objective 2 –– To what extent is the body FA profile of crayfish influenced by that of their diet? –– we used a single fixed-factor model with three levels (detritus, invertebrate, pellet; corresponding to our three experimental laboratory diet treatments) to compare crayfish body FA composition among treatments at the end of the 70-day experiment. For our second question –– Which FAs become

TABLE 1 Description of the linear mixed-effects model fitted to the crayfish growth data

Model	Formula	Description
Null 1	$m_{i,t} = \beta + b_i + \epsilon_{i,t}$ $i = 1, \cdots, m, t = 1, \cdots, n$ $b_i \sim N(0, \sigma_1^2)\, \epsilon_{i,t} \sim N(0, \sigma^2)$	Mass of subject i measured at time t is equal to the population mean mass over the observation period (β) + the deviation from that mean imposed by subject i (b_i) + error ($\epsilon_{i,t}$). m is the number of subjects in the experiment ($m = 120$ at Day 0), while $n = 11$ is the number of observations made over the 70-day experiment
Null 2	$m_{i,t} = \beta + b_i + (\beta_1 + b_{1i})d + \epsilon_{i,t}$ $i = 1, \cdots, m, t = 1, \cdots, n$ $b_i \sim N(0, \sigma_1^2)\, b_{1i} \sim N(0, \sigma_2^2) \epsilon_{i,t} \sim N(0, \sigma^2)$	As Null 1 but now including a fixed parameter (β_1) for the effect of day, d, and a random parameter (b_{1i}) for the deviation from the population mean rate of mass increase due to subject i
Model 1	$m_{i,t} = \beta + b_i + (\beta_1 + b_{1i})d + \beta_{2j} + \epsilon_{i,t}$ $i = 1, \cdots, m, t = 1, \cdots, n\, j = 1, 2$ $b_i \sim N(0, \sigma_1^2)\, b_{1i} \sim N(0, \sigma_2^2) \epsilon_{i,t} \sim N(0, \sigma^2)$	As for Null 2 but now including contrasts (β_{2j}) for the fixed effects of diet. Two treatment contrasts are included to describe the departure of diets *invertebrates* and *pellets* from *detritus*
Model 2	$m_{i,t} = \beta + b_i + (\beta_1 + b_{1i})d + \beta_{2j} + \beta_{3j}d + \epsilon_{i,t}$ $i = 1, \cdots, m, t = 1, \cdots, nj = 1, 2$ $b_i \sim N(0, \sigma_1^2)\, b_{1i} \sim N(0, \sigma_2^2) \epsilon_{i,t} \sim N(0, \sigma^2)$	As for Model 1 but now including further contrasts (β_{3j}) for the interaction effect between day and diet. The additional two contrasts are treatment contrasts as described in Model 1

over- and under-represented in crayfish bodies, relative to their dietary FA profiles as a result of digestion and biosynthesis? –– we calculated the direction and magnitude of FA modification as the 10% trimmed mean of the $\log_{10}$-transformed ratio between the proportions of FA in crayfish tissue to the proportion in their food. These were calculated by computing all permutations of ratios between replicate crayfish tissues and food samples following methods outlined previously (e.g., see Iverson et al., 2004; Thomas et al., 2020). Briefly, a food treatment containing 10 replicate crayfish and three replicate samples of the food used in the treatment resulted in 30 ratios per FA, from which the trimmed mean was calculated.

2.5.3 | Objective 3: Comparison of FA profiles among river–floodplain habitats

In order to compare FA profiles of diets and body composition of wild crayfish we used a two-factor model; habitat (fixed; two levels: wetland and river) and source (fixed; two levels: diet and body). Non-metric multi-dimensional scaling (NMDS) was used to visualise crayfish FA profiles in multivariate space using R/VEGAN (Oksanen et al., 2013; R Core Team, 2018). Diet and body FA profiles measured under the experimental (Objective 2) and field (Objective 3) settings were plotted on the same ordination, which facilitated interpretation of the field FA profiles.

3 | RESULTS

3.1 | Objective 1: Crayfish growth on controlled diets

The most likely model of crayfish growth under our experimental conditions was Model 2, which was the model including the interaction between *diet* and *day* (Table 2). The null models had AIC weights of approximately zero (Table 2). The parameters for Model 2 are presented in Table S4. Diagnostic plots of residuals indicated that Model 2 well-fitted the data (Figure S2). Using Nakagawa's R^2 (Nakagawa & Schielzeth, 2013), the marginal R^2 was 0.4 (fixed effects only) whereas the conditional R^2 was 0.94 (fixed + random effects). There was a large amount of variation in growth rate among subjects (Figures 1 and S3), as indicated by the high conditional R^2 relative to the marginal R^2, as well as the high value of σ_1 (*SD* of subject intercepts) relative to residual error (Table S4). Growth rates of crayfish varied significantly and strongly among treatments, with the variation in growth rate among diets being described by the inequality 'pellets > invertebrates > detritus' (Figure 1). Over the 70-day feeding trial, growth of crayfish on detritus was negligible, whereas crayfish feeding on pellets more than doubled in mass (Figure 1).

TABLE 2 Performance statistics for the models in the candidate set, sorted in increasing order by their Akaike information criterion (AIC); decreasing log-likelihood (Log(L)). $\Delta_i = \text{AIC}_i - \min(\text{AIC})$ is the AIC model rank; and w_i is the Akaike weight of model i, interpreted as the approximate probability that Model i is the best model in the candidate set (Burnham & Anderson, 1998)

Model	AIC	Δ_i	w_i	log(L)
Model 2	−3206.2	0.0	1.0	1613.2
Model 1	−3041.4	164.8	0.0	1528.8
Null 2	−3041.4	164.8	0.0	1526.7
Null 1	−1821.3	1384.9	0.0	913.6

3.2 | Objective 2: Biosynthesis of FAs by crayfish on controlled diets

3.2.1 | Overall crayfish FA profiles

A total of 51 FAs were detected among laboratory-fed and wild crayfish (Figure S4). The seven most abundant FA comprised >68% of the total profile (16:0, 20.1 ± 0.3%; 18:1ω9 [OA], 16.8 ± 0.9%;

18:2ω6 [LIN], 8.9 ± 1.1%; 18:0, 7.9 ± 2.8%; 18:3ω3 [ALA], 5.0 ± 0.4%; 20:4ω6 [ARA], 4.8 ± 0.4%; and 20:5ω3 [EPA], 4.6 ± 0.4%). Saturated, polyunsaturated and monounsaturated FA contributed 37.7 ± 0.8%, 34.0 ± 1.0% and 28.2 ± 1.1%, respectively, to the total crayfish FA profile.

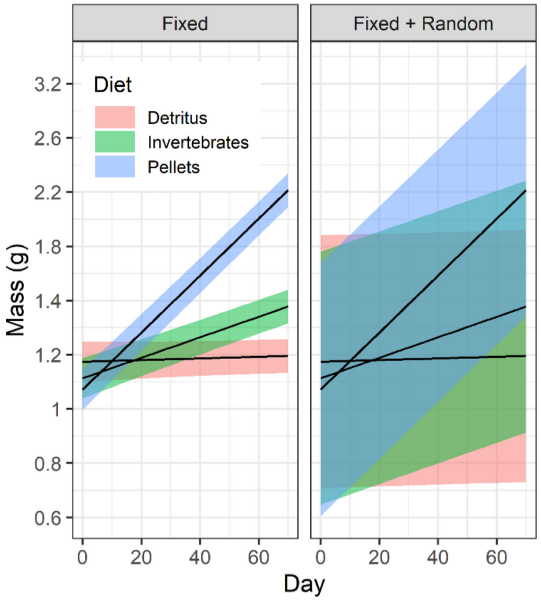

FIGURE 1 Fitted model (Model 2; ± 95% CI) of crayfish change in mass (note log-scale) as a function of diet and time since the start of the feeding experiment. Confidence intervals in the left panel indicate uncertainty around fixed effects of *day* and *diet* only, whereas those on the right indicate uncertainty due to both fixed and random (*subject*) effects

3.2.2 | Question 1: To what extent is the body FA profile of crayfish influenced by that of their diet?

At the end of our 70-day feeding trial FA profiles of crayfish bodies varied significantly among the three diet treatments (pseudo-$F = 44.5$, $p = 0.001$; Figure 2a, Table 3). The proportion of LIN within crayfish bodies contributed strongly to dissimilarity among treatments (SIMPER; Table 4, Figure 2b) and mirrored the inequality observed in the growth experiment and a priori diet analyses of pellets (20.5 ± 0.3%) > invertebrates (9.8 ± 0.3%) > detritus (5.6 ± 0.1%) (Table 5). Proportion of POA and OA FAs also contributed >5% to dissimilarity between crayfish FA profiles at the end of the experiment (SIMPER; Table 4, Figure 2b).

3.2.3 | Question 2: Which FAs become over- and under-represented in crayfish bodies, relative to their dietary FA profiles as a result of digestion and biosynthesis?

Crayfish feeding on pellets and detritus exhibited greater modification of FA profiles as a result of digestion and biosynthesis than those feeding on invertebrates (Figure 2a). When compared with the FA profile of their diets, pellet-fed crayfish preferentially assimilated greater proportions of four FAs: 20:4ω6 (ARA), 20:5ω3 (EPA) 18:1ω9 (OA) and 16:1ω7 (POA; Figure 3). By contrast, crayfish fed detritus and invertebrates preferentially assimilated higher proportions of a greater number of FAs (22 and 25, respectively), although for crayfish fed on invertebrates, more FA were in similar proportions to their diet. Detritus- and

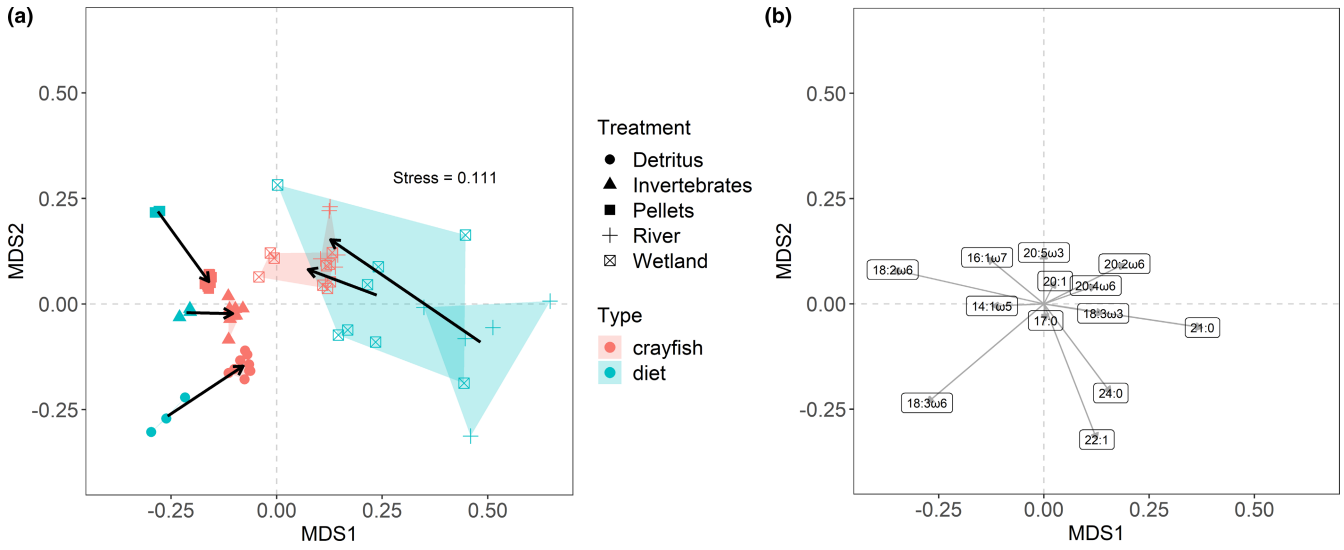

FIGURE 2 NMDS plots of (a) arcsine-square-root-transformed proportions of FAs in crayfish and their diets. Arrows show direction of modification of dietary FAs by crayfish. Vectors are placed within the centroids of each group of points and were added to visualise the multivariate shift in Euclidean distance (similarity) of FA profiles of diets to the resulting crayfish FA profiles; this shows the relative magnitude and direction of the shift in NMDS space of the crayfish modification of dietary FAs (polygons are calculated convex hulls for each grouping of treatment and type) and (b) positions of FAs within the NMDS space showing only those FAs that contributed >5% to dissimilarity (SIMPER) among treatments and types

TABLE 3 PERMANOVA results for laboratory crayfish FA profiles (Objective 2), and wild crayfish diet and body FA profiles (Objective 3)

Objective	Factor	*df*	Pseudo-*F*	*P*
2. Laboratory crayfish FA profiles	Treatment	2, 28	44.5	0.001
3. Wild crayfish diet and body FA profiles	Habitat	1, 27	2.0	0.044
	Source	1, 27	8.2	0.001
	Habitat × Source	1, 27	2.3	0.028

TABLE 4 Similarity percentage analysis (SIMPER) of FAs contributing >5% to dissimilarity between laboratory-fed crayfish FA profiles (Objective 2), and the diets and crayfish profiles of wild river- and wetland-collected crayfish (Objective 3). Fatty acids arachidonic (ARA), alpha-linolenic (ALA), linoleic (LIN), eicosapentaenoic (EPA), palmitoleic (POA) and oleic (OA) are included in parentheses beside the relevant lipid names

			Average		Contribution
Objective	Groups	FA	abundance		%
2. Laboratory-fed crayfish	Detritus and pellet average dissimilarity = 23.29		**Detritus**	**Pellet**	
		18:2 ω6 (LIN)	13.69	26.95	9.63
		16:1 ω7 (POA)	5.72	14.27	6.21
		17:00	12.11	4.56	5.49
		18:1 ω9 (OA)	17.53	25.07	5.48
		18:3 ω6	12.04	4.60	5.41
		18:3 ω3 (ALA)	17.24	10.18	5.14
	Detritus and invertebrate average dissimilarity = 17.84		**Detritus**	**Invertebrate**	
		16:1 ω7 (POA)	5.72	12.84	6.39
	Pellet and invertebrate average dissimilarity = 15.45		**Pellet**	**Invertebrate**	
		18:2 ω6 (LIN)	26.95	18.26	9.62
		14:1 ω5	2.04	7.06	5.57
3. Wild crayfish gut contents	River and wetland average dissimilarity = 38.83		**River**	**Wetland**	
		21:0	11.8	3.91	8.23
		20:4 ω6 (ARA)	14.30	10.69	7.36
		20:1(isomers)	0.00	8.00	6.07
		20:2 ω6	5.12	4.85	5.79
		24:0	12.77	5.53	5.59
		22:1(isomers)	5.21	4.10	5.47
		20:5 ω3 (EPA)	2.63	7.58	5.15
3. Wild crayfish	River and wetland average dissimilarity = 17.55		**River**	**Wetland**	
		18:2 ω6 (LIN)	0.59	6.07	7.57
		20:4 ω6 (ARA)	12.07	16.43	6.65
		18:1 ω9 (OA)	31.02	26.13	6.03
		20:5 ω3 (EPA)	17.87	14.21	5.49

invertebrate-fed crayfish preferentially assimilated the saturated FAs caprylic acid (8:0) and margaric acid (17:0), and ω6 LC polyunsaturated FA 20:4ω6 (ARA) in the highest proportions relative to their diet. Detritus-fed crayfish assimilated greater proportions of ALA and gamma-linolenic acid (18:3ω6; GLA) than other treatments and invertebrate-fed crayfish assimilated higher proportions of DHA than crayfish fed detritus or pellets. Among all dietary treatments, LIN was found in higher proportions in foods than in crayfish tissue (Figure 3).

3.3 | Objective 3: Comparison of FA profiles among river–floodplain habitats

Fatty acid profiles of wild crayfish and their diets differed significantly between wetland and river habitats (pseudo-F = 2.0, p = 0.044) and between crayfish body tissue and their diets (pseudo-F = 8.2, p = 0.001), although there was a significant interaction (pseudo-F = 2.3, p = 0.028; Table 3). Pairwise comparisons indicated that FA profiles of both crayfish (t = 1.78, p = 0.027)

TABLE 5 Mean contribution of FA classes (%) to total lipid profiles of diets and crayfish

	Detritus			Invertebrate			Pellet			River			Wetland		
Variables	Diet	± SE	Crayfish	± SE	Diet	± SE	Crayfish	± SE	Diet	± SE	Crayfish	Diet	± SE	Crayfish	Diet	± SE	Crayfish	± SE
∑SFA	43.7	3.9	43.9	1.4	37.4	0.9	36.5	0.4	32.3	0.1	31.8	0.3	60.6	2.6	39.3	1.4	49.2	4.6	38.5	0.8
∑MUFA	13.2	4.0	15.5	0.4	26.8	0.3	29.4	0.9	23.3	0.2	30.2	0.6	15.3	3.8	36.4	0.8	25.2	3.1	32.5	1.5
∑PUFA	43.0	4.5	40.6	1.2	35.9	0.9	34.1	0.8	44.4	0.2	38.0	0.7	24.2	1.6	23.6	1.3	25.6	2.8	28.8	1.9
LIN	6.4	3.1	5.6	0.1	17.1	0.2	9.8	0.3	32.3	0.3	20.5	0.3	0.0	0.0	0.1	0.0	0.9	0.9	3.3	1.6
ALA	4.6	0.2	8.9	0.7	5.2	0.0	5.0	0.3	3.7	0.0	3.1	0.1	10.1	1.1	2.6	0.2	8.3	2.0	4.8	0.4
ARA	0.6	0.3	4.5	0.6	1.7	0.0	4.1	0.2	1.1	0.0	2.6	0.2	7.8	2.4	5.1	1.2	5.4	1.8	8.1	0.6
EPA	1.3	0.1	3.2	0.5	1.5	0.1	3.4	0.2	1.7	0.0	3.1	0.2	1.0	1.0	9.7	1.1	2.7	0.8	6.1	0.4
DHA	5.9	1.9	1.5	0.2	0.5	0.3	1.1	0.1	4.4	0.2	2.7	0.1	0.0	0.0	2.5	0.2	2.4	0.9	2.5	0.3
POA	1.2	1.6	1.0	0.1	6.8	0.0	5.0	0.4	3.1	0.0	6.1	0.4	0.0	0.0	2.6	0.4	1.6	0.7	3.9	0.3
OA	4.9	1.8	9.1	0.1	11.0	0.1	15.0	0.4	16.2	0.2	18.0	0.4	11.4	1.2	26.6	1.0	14.3	1.1	19.6	1.4

and their diets ($t = 1.41$, $p = 0.026$) differed significantly between habitats and that within each habitat, FA profiles of crayfish differed from their diets (river: $t = 2.96$, $p = 0.007$; wetland: $t = 1.67$, $p = 0.001$). Dissimilarity between FA profiles of river and wetland crayfish diets was driven by higher proportions of saturated FA 21:0 and 24:0 in the guts of river crayfish and higher proportions of EPA in diets of wetland crayfish (SIMPER; Table 4). In contrast to experimental foods, diets of wild crayfish were higher in proportion of the long-chain (>20 C) polyunsaturated FA ARA (river 7.8 ± 2.4% and wetland 5.4 ± 1.8%; Table 4). LIN was not detected from the gut contents of any river crayfish and was detected from the gut contents of only one wetland crayfish, where it comprised 8.17% of total FAs. Differences between wetland and river crayfish body FA profiles were driven primarily by the proportion of ω6 polyunsaturated FAs LIN and ARA, which were higher among wetland crayfish (SIMPER; Table 4).

4 | DISCUSSION

4.1 | Growth

We hypothesised that the pattern of growth across our dietary treatments would be consistent with the inequality 'pellets > invertebrates > detritus', and our results support this hypothesis. We show clear differences in the growth of crayfish fed on different quality diets, with individuals given high-quality balanced diet, particularly rich in the polyunsaturated ω6 FA LIN, more than doubling in mass compared to those fed solely on detritus, for which growth was negligible.

Despite strong effects on growth, diet quality did not significantly influence survival. The addition of LIN to the diets of Crustacea has long been known to improve growth rates of marine aquaculture species (Read, 1981; Shewbart & Mies, 1973), although trophic and metabolic dynamics among freshwater Crustacea are less studied. In our experiment, proportions of LIN were significantly higher in our pellet treatment and growth rates of these crayfish were significantly higher than growth rates of their counterparts fed detritus or invertebrates. The monoeneoic FA 18:1ω9 (OA) also was associated with high growth in crayfish. Although the role of OA in freshwater food webs is not well-understood, being mostly associated with fungi, it is consumed in large quantities during the growth of farmed teleost fish species, and particularly so by female fish during the formation of roe (Henderson & Almatar, 1989; Henderson et al., 1984). Among marine fish, monounsaturated FAs in general are an important energy source, since they are preferentially oxidised compared with long-chain saturated FAs (Sidell et al., 1995). Our results are consistent with crayfish growth responses reported elsewhere that have shown that diets rich in LIN and OA perform as well as diets containing high levels of ω3 highly unsaturated FAs EPA and DHA for crayfish grown indoors and lacking natural food items (Thompson et al., 2010).

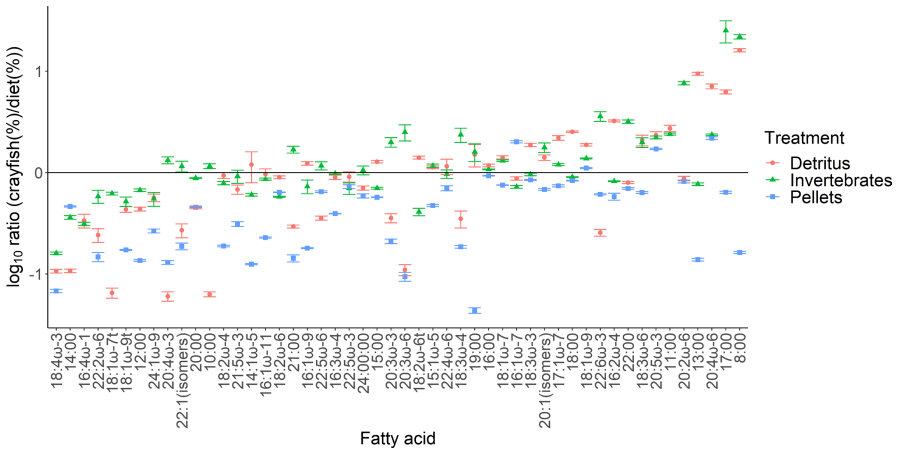

FIGURE 3 Log_{10} ratio of FA proportion (%) in crayfish tissues/FA proportion (%) in crayfish food in the trophic modification experiment, organised by ranking of the ratios. Ratios above 0 (black line) are found in higher proportions in crayfish. Error bars are *SE*

4.2 | Patterns of fatty acid modification and integration

At the end of our 70-day trial, integrated FA profiles of crayfish fed different foods were strongly differentiated from each other, consistent with studies of marine crustaceans (Thomas et al., 2020). However, in contrast to Thomas et al. (2020) who reported that marine crabs modified dietary FAs in a similar way for each of their distinct experimental diets (suggesting a consistent strategy for lipid metabolism), we found that freshwater crayfish fed high-quality pellets were selectively enriched in only four FAs; two long-chain polyunsaturated FAs 20:4ω6 (ARA) and 20:5ω3 (EPA), and two monounsaturated FAs 18:1ω9 (OA) and 16:1ω7 (POA), suggesting that proportion of these molecules may be particularly important for their somatic growth. Proportion of ARA and EPA in tissues of other aquatic animals have been associated with increased growth and survival (e.g., Li et al., 2020; Nhan et al., 2020), and as discussed previously, monounsaturated FAs, such as POA and OA, are known to be important energy sources for fish (Tocher, 2003) and have been associated with ontogenetic changes to metabolism to maximise growth or for periods of fasting (Chaguaceda et al., 2020).

By contrast, crayfish fed detritus and invertebrates preferentially assimilated higher proportions of a greater number of FAs, suggesting greater potential dietary limitation than for pellet-fed crayfish. Detritus-fed crayfish assimilated higher proportions of polyunsaturated FAs alpha-linolenic (18:3ω3; ALA) and gamma-linolenic acid (18:3ω6; GLA) and crayfish fed the invertebrate diet assimilated higher proportions of docosahexaenoic acid (22:6ω3; DHA) relative to their diets than crayfish in detritus or pellet treatments. These results are not surprising, since the importance of these molecules for survival and growth is also well-documented among a wide range of ecosystems and organisms (Brett et al., 2009; Galloway et al., 2014). Laboratory crayfish FA profiles strongly reflected their dietary resources, and proportions of essential FA EPA and ARA were consistently higher in crayfish than in their diets, suggesting physiological control of proportions and anabolism from shorter-chain molecules, such as ALA and LIN, a pattern observed among some fish species in other ecosystems (Happel et al., 2016).

The proportion of DHA was lower in crayfish than their diets among detritus- and pellet-fed crayfish, yet we expect that this was because it was provided in excess to their requirements, consistent with assimilation relationships reported elsewhere (Thompson et al., 2010). Docosahexaenoic acid proportion in our experimental pellet and detritus diets was very high (means 4.4 ± 0.2% *SE* and 5.9 ± 1.9% *SE*, respectively) compared to the invertebrate diet and to gut contents of wild crayfish (<3%). The high proportion of DHA within our detritus diet treatment was surprising, since typically detritus lacks LC-PUFAS (e.g., Guo et al., 2018; Lau et al., 2009). Our detritus comprised *E. camaldulensis* leaves soaked in mesocosm tanks filled with river water for three weeks to mimic benthic litter under natural conditions. It is likely that microbial activity of fungi and algae in mesocosms during this period produced the elevated DHA levels that we detected in our detrital pellets (e.g., reflecting synthesis of DHA by fungi and algae; Bajpai et al., 1991; Taipale et al., 2013).

Compared with their foods, all laboratory-fed crayfish had lower proportions of the long-chain FA precursor linoleic acid (LIN; 18:2ω6), which may suggest that freshwater crayfish use these FAs to biosynthesise longer-chain ω6 FA, such as ARA. Thus, it is possible that low proportions of LIN with detritus diets could be partly responsible for lower proportions of ARA, and subsequent poor growth among detritus-fed crayfish. LIN is widely recognised for its importance among animals for cell physiology, immunity and reproduction (Brett & Müller-Navarra, 1997; Guo et al., 2016; Jardine et al., 2020). Bacteria, protozoa and plants are known to synthesise LIN, but humans are incapable of synthesising LIN themselves, and it was long thought that animals generally were incapable of de novo synthesis. As such, LIN often is referred to as an 'essential' FA, since it was thought that animals must derive it from their diet alone. However, as more studies have examined animal metabolism, researchers discovered that some animals, including insects (Louloudes et al., 1961) and more recently crustaceans (Malcicka et al., 2018), are capable of de novo synthesis of LIN. It remains unclear as to why some organisms can synthesise LIN and some cannot, or if those that can, do so in sufficient quantities to meet their specific requirements, and this remains an active area of research (Malcicka et al., 2017). Our results

suggest that if crayfish are capable of LIN biosynthesis, it is likely in small quantities only, since the inequality of 'LIN pellets > invertebrates > detritus' persisted from diets to body tissue. We demonstrate that FA profiles of crayfish are shaped strongly by their diet, and that selective integration of high-quality FAs from basal resources rich in these micronutrients can lead to higher proportions of these FAs in crayfish tissues.

4.3 | Wild crayfish FA profiles and implications for food webs

The fatty acid profiles of wild crayfish diets differed significantly between wetland and river habitats, and consistent with our laboratory experiments, crayfish body FA profiles also differed between habitats. The proportion of LIN, which was associated with high growth among experimental crayfish was not detected from the gut contents of riverine crayfish, and only from the gut contents of one wetland crayfish. Based on our laboratory results, it is possible that somatic growth of wild crayfish could be limited by a lack of dietary LIN. However, since proportion of ARA in wild crayfish diets from both wetland and river habitats was substantially higher than the proportion of ARA in any of our experimental diets, we propose that wild crayfish may have less reliance on biosynthesis of ARA from LIN than we had in our laboratory experiment, sourcing ARA instead directly from prey.

Fatty acid profiles of crayfish differed between habitats, with differences driven primarily by higher proportions of ARA and its shorter-chain ω6 biochemical precursor, LIN, among wetland crayfish. These results have nutritional implications for riverine predators that as a direct result of river regulation and the reduction of large floodplain inundation events, have reduced access to floodplain prey. Previous work has shown that floodplain habitats can provide higher quality basal food resources for primary consumers than riverine habitats (McInerney et al., 2020), and here we identify one mechanism by which that may be extended to subsequent trophic levels (see Figure S5). Riverine crayfish contained the lowest proportion of LIN among all crayfish in the study. Thus, based on results from our laboratory experiments, somatic growth of wild river crayfish populations may be limited by their food, with cascading implications for fish populations that are reliant on crayfish for their primary food source. Although the optimum ratio of ALA:LIN for somatic growth of marine fish is much-studied (e.g., Wu & Chen, 2012), requirements for freshwater species are less well-known. However, from the limited research that has been conducted, the concentration of LIN in the diets of the Murray–Darling Basin's apex fish predator, the Murray cod, were significant for obtaining increased somatic growth (De Silva et al., 2002; Francis et al., 2006).

Despite much lower proportions of LIN in their tissues than our experimental crayfish, wild crayfish contained high (albeit variable in comparison to experimental diets) proportions of the long-chain ω3 polyunsaturated FAs EPA and DHA in their diets and in their tissue. Diatoms are a rich source of EPA and DHA (Peltomaa et al., 2019), and our results may reflect high availability of algal food resources in the wild, suggesting that these molecules may not be limiting to somatic growth of wild populations. These LC-PUFAs can be obtained directly from their diet or converted from dietary shorter-chain PUFAs, such as ALA (Murray et al., 2014), which was recorded in similar proportions across our experimental diet treatments, and in higher proportions among the diets of wild crayfish. ALA also was retained in higher proportions by detritus-fed crayfish, possibly reflecting selective retention of algal ω3 polyunsaturated FA from biofilms on aged detritus (Kühmayer et al., 2020) by crayfish fed the poor quality diet. ALA also is abundant in terrestrial plants (Guo et al., 2017, 2020) and the high proportion within the diets of wild crayfish could suggest some level of dietary reliance on macrophytes which are a particularly rich source of ALA in these habitats (McInerney et al., 2020).

Our results provide evidence that floodplain wetlands can contain higher-quality food resources for crayfish compared with river channel habitats, providing support for the Flood Pulse Concept, and accentuating the importance of floodplain–river connectivity for river management. Recognising that a variety of biotic and abiotic elements (e.g., competition, habitat, life-history traits, spawning triggers) may limit riverine populations, we propose that high-quality basal resources have the potential to support a greater biomass of apex predators, and conversely, a poor-quality diet may lead to primary consumer FA profiles that support lower overall biomass of apex predators in the wild. Our study also provides evidence for the conversion of shorter-chain essential FAs by *C. destructor* to compensate for a lack of long-chain FAs in their diet. We provide a necessary first step for improving our understanding of the role of FAs in the transfer of energy between trophic levels in lowland river food webs. Our study also makes a constructive link between experimental laboratory diets and those of wild populations for a keystone species and the implications for higher-order consumers.

5 | CONCLUSIONS

Here we provide FA profiles for a key species among lowland river food webs. Freshwater crayfish represent a primary food source for large-bodied Australian fishes, and we supply important information relating to the quality of their diets and growth. Although it is increasingly accepted that FA profiles of consumers reflect their diets, controlled studies that trace FA profiles of diets to consumers are lacking, particularly among invertebrates. Recognising the limitations of inference from a 70-day laboratory experiment and a temporally limited field collection, here we provide the necessary first step for improving our understanding of relationships between basal resources and consumers. Further work is needed to trace the transfer of essential nutrients from secondary consumers to higher-order predators, but we provide a useful baseline on which to build future research.

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CONFLICT OF INTEREST

The authors have no conflict of interest to disclose.

AUTHOR CONTRIBUTIONS

Conceptualisation: P.J.M., R.J.S, G.N.R. Developing methods: P.J.M., R.J.S, M.E.S., G.N.R, C.D.D, J.A. Data analysis: P.J.M., R.J.S, M.E.S. Preparation of figures and tables: P.J.M., R.J.S, M.E.S. Conducting the research, data interpretation, writing: P.J.M., R.J.S, M.E.S., G.N.R, C.D.D, J.A.

DATA AVAILABILITY STATEMENT

Data are available from the corresponding author upon request.

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