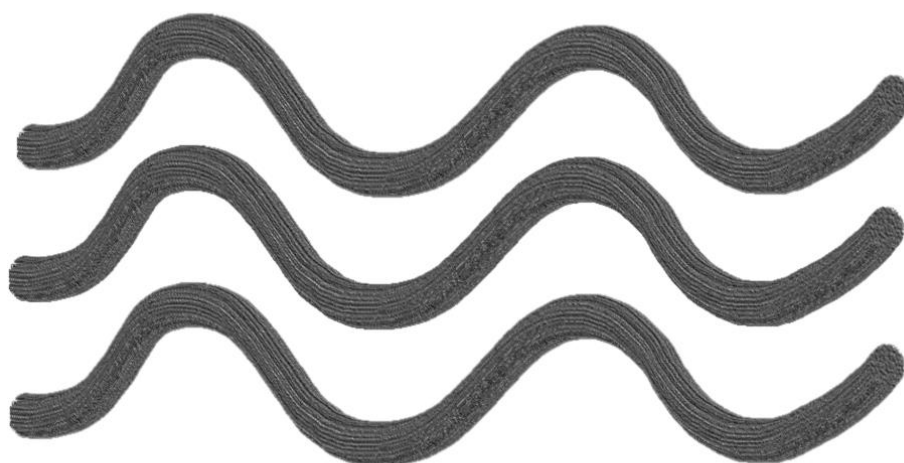


Freshwater inflows to estuaries: Terrestrial resource inputs and planktonic food web responses



Ellery B. Johnson

A thesis in fulfilment of the requirements for the degree of
Doctor of Philosophy

June 2022

Freshwater and Estuarine Research Group
School of Life Sciences
University of Technology Sydney

Certificate of original authorship

I certify that the work in this thesis has not previously been submitted for a degree or as part of the requirements for a degree. I also certify that this thesis has been written by me. Any help received throughout the research process and preparation of this thesis has been acknowledged. In, addition, I certify that all information sources and literature used are indicated in the thesis. This research was supported by the Australian Government Research Training Program.

Production Note:

Signature removed

Signature: prior to publication.

Date: June 5, 2022

Acknowledgements

Throughout this journey I have been surrounded by supportive people who value learning, education, community, and the environment. This work has been helped and supported by so many people across the last 5 years and I hope I cross paths with you all again in the future. If it takes a village to raise a child, it takes a strong support network and caring people to raise a researcher.

The greatest thanks of all are owed to Prof. Simon Mitrovic. I could not have completed this project without your knowledge, guidance, support, enthusiasm, and friendship along the way. Being afforded the freedom to make my own mistakes has been the greatest gift you have given me. The opportunities and networks you have shown me since beginning my Honours project in 2015 have genuinely changed my life. I would not be who I am today without your support.

To my co-supervisors Wade Hadwen, James Hitchcock and Craig Boys, thank you for your expertise, your edits and your friendship. Your specialist knowledge made the exploration of new concepts less daunting and lonely. I look forward to working with you all again in the future.

I would also like to acknowledge NSW DPIE Water for their financial and organisational support of the project, in particular Doug Westhorpe who was instrumental in data sharing and early field trips and the DPIE lab at Wollongbar for the analysis of samples in the early stages of this project.

To my co-authors and collaborators, Tim Smith, Troy Gaston, Abeeha Khalil, Terrence Rogers, Jordan Facey, Unni Kuzhiumparambil and Stew Fielder thank you for your help in analysis and discussion of results in more unfamiliar territory.

To the technical assistants and tinkerers – in particular Helen Price, Doug Braathen, Taya Lapshina, Nigel Coombes, Luke Cheviott and Graham Housefield – thanks for your support ensuring field work, experiments and analysis ran smoothly...even if they didn't at times.

To the plethora of people who helped me in the field, Finlay Johnson, Elliot Pearson, Paloma Matis, Laura Michie, Sinead McLaughlin, Sidonie McLaughlin, John Nowlan, Terrence Rogers, Lemeki (Big Tommy) Vuetaki, Allyson Theseria, Emily Scott and Sophie

Montgomery. Each field trip has a story, from breaking down in the middle of a river to boat trailers leaping off the tow ball, thank you for being a part of the journey.

Special thanks must also go to Pat Sinclair for allowing me to use her farmhouse on Oxley Island as accommodation. What a treat this was.

To the friends who mentored and supported me through this process, in particular, Lisa Roberts, Alex Thompson, Kirsty Milner, An Tran, Caitlin Lawson, Samantha Goyen and Paloma Matis who acted as a mixture of big-sisters and cool aunties through this journey. Thanks also to the friends I have made at UTS, those in the FERG group; Laura Michie, Lauren O'Brien, Matt Balzer, Jordan Facey, Terrence Rogers, Jarrod Walton, and those outside; Peter Irga, Fraser Torpy, Nine La Rune, Scott Chadwick, Charlotte Lindsay, Joel Steele, Nathan Williams, Kieran Young, Thomas Hawthorne, Trent Haydon, Gemma Gillette – bumping into nice people in corridors makes the work worthwhile.

To my friends outside of science, Bimbi, Charles and Mary, Jack and Claire (thanks for the maps Claire!), Crackers and Jess, Cheese, Quinn, Steph and Toby, Dec Byrnes, thanks for being there for me. Whether it was a kick of the footy, an afternoon schooner, a walk around the park or a chat on the phone, your friendship means a great deal to me.

To my family, you are everything to me. Plain and simple. I love you all dearly.

Lastly, Sinead McLaughlin. I love you and I would not have come close to finishing this without you. You make me a better person every day. My successes are your successes.

Acknowledgement of Country

I acknowledge the people of the Eora Nation, where I live and learn, as well as the Biripi, Worimi, Kamilaroi and Darkinjung Nations on whose land this knowledge was also developed.

My respect is owed to Elders past, present and emerging and I acknowledge them as the traditional custodians of knowledge in this great land. Together we work towards a brighter and reconciled future for all.

Place, people, and knowledge are inseparable. In listening we learn.

Preface

This thesis contains 6 chapters. Chapters 2-5 are written as self-contained journal articles in preparation for submission to peer reviewed journals. They are presented in a format similar to independent manuscripts and therefore some repetition may occur between chapters.

Where these chapters do cross-reference each other relevant chapter numbers are provided.

To reduce further unnecessary repetition a single reference list has been provided at the end of this thesis.

This thesis is a compilation of my own work. I conceptualised the research, obtained and processed samples, conducted data analysis and wrote the chapters contained in this thesis. While guidance, support and assistance were provided by supervisors and co-authors this thesis is written using the singular “I” rather than the plural “we”. The titles of each chapter and the contributions of co-authors are listed below.

Chapter 2: Responses of estuarine zooplankton to freshwater inflows: a comparison between a regulated and unregulated estuary

Ellery B. Johnson, James N. Hitchcock, Wade L. Hadwen, Douglas P. Westhorpe and Simon M. Mitrovic

JH – conceptual advice and guidance

Production Note:
Signature removed prior to publication.

WH – conceptual advice and guidance

Production Note:
Signature removed prior to publication.

DW – conceptual advice, guidance, and field assistance

Production Note:
Signature removed prior to publication.

SM – conceptual advice, guidance, and field assistance

Production Note:
Signature removed prior to publication.

Chapter 3: Fine scale food web responses to a freshwater inflow event in a small regulated Australian estuary

Ellery B. Johnson, James N. Hitchcock, Terrence A. Rogers, Abeeha Khalil, Wade L. Hadwen, and Simon M. Mitrovic

JH – conceptual advice and guidance

Production Note:
Signature removed prior to publication.

TR – algal analysis and advice

Production Note:
Signature removed prior to publication.

AK – bacterial analysis and advice

Production Note:
Signature removed prior to publication.

WH – conceptual advice and guidance

Production Note:
Signature removed prior to publication.

SM – conceptual advice, guidance and field assistance

Production Note:
Signature removed prior to publication.

Chapter 4: Inflow events drive resource use changes for mesozooplankton in a regulated estuary

Ellery B. Johnson, Wade L. Hadwen, James N. Hitchcock, Jordan A. Facey, Tim Smith, Troy F. Gaston, and Simon M. Mitrovic

WH – conceptual advice, guidance, stable isotope assistance

Production Note:
Signature removed prior to publication.

JH – conceptual advice and guidance

Production Note:
Signature removed prior to publication.

JF – algal analysis and advice

Production Note:
Signature removed prior to publication.

TS – stable isotope assistance and advice

Production Note:
Signature removed prior to publication.

TG – stable isotope assistance and advice

Production Note:
Signature removed prior to publication.

SM – conceptual advice, guidance and field assistance

Production Note:
Signature removed prior to publication.

Chapter 5: Can zooplankton fed allochthonous carbon diets support and sustain juvenile fish?

Ellery B. Johnson, Craig A. Boys, D. Stewart Fielder, Wade L. Hadwen, Unnikrishnan Kuzhiumparambil, James N. Hitchcock, and Simon M. Mitrovic

CB – conceptual advice, guidance, and lab assistance

Production Note:
Signature removed prior to publication.

SF – conceptual advice and guidance

Production Note:
Signature removed prior to publication.

WH – conceptual advice, guidance, and stable isotope assistance

Production Note:
Signature removed prior to publication.

UK – essential fatty acid analysis and advice

Production Note:
Signature removed prior to publication.

JH – conceptual advice and guidance

Production Note:
Signature removed prior to publication.

SM – conceptual advice and guidance

Production Note:
Signature removed prior to publication.

Other papers and reports published during my candidature but not forming part of this thesis:

Hammill, E., Johnson, E., Atwood, T.B., Harianto, J., Hinchliffe, C., Calosi, P. and Byrne, M., 2018. Ocean acidification alters zooplankton communities and increases top-down pressure of a cubozoan predator. *Global Change Biology*, 24 (1), p. e128-e138.

O'Brien, L., Johnson, E., Balzer, M., Rogers, T., Michie, L., Hitchcock, J., Hadwen, W., Westhorpe, D., Mitrovic S., 2020. Carbon and nutrient transport, food webs and the effectiveness of high flow protection and end-of-system flow rules; An assessment of the current flow rules on the Gwydir, Macquarie, Williams and Wyong Rivers, NSW, Report to NSW DPIE. May 2020

Roberts, L., Kutay, C., Melbourne-Thomas, J., Petrou, K., Benson, T.M., Fiore, D., Fletcher, P., Johnson, E., Silk, M., Taberner, S. and Filgueira, V.V., 2021. Enabling enduring evidence-based policy for the Southern Ocean through cultural arts practices. *Frontiers in Ecology and Evolution*, 9, p. 284

Contents

Certificate of original authorship	i
Acknowledgements.....	ii
Acknowledgement of Country	iv
Preface.....	v
Contents	vii
List of Figures	xi
List of Tables	xvi
List of Abbreviations	xviii
Ethics Statement.....	xix
Abstract.....	xx
Chapter 1: Introduction	1
1.1 Research Scope and Significance.....	1
1.2 Freshwater inflows and estuaries	3
1.3 Estuarine productivity and resource inputs	6
1.4 Basal energy sources and inflows	7
1.5 Consumer responses to inflows.....	9
1.6 Reducing impacts of river regulation on estuaries	10
1.7 Study Sites and WSPs	11
1.8 Research Aims.....	15
Chapter 2: Responses of estuarine zooplankton to freshwater inflows: a comparison between a regulated and unregulated estuary	18
2.1 Abstract	18
2.2 Introduction	18
2.3 Methods.....	21
2.3.1 Site Description.....	21

2.3.2	Sampling	23
2.3.3	Statistical analysis.....	24
2.4	Results	25
2.4.1	Hydrology and resource delivery.....	25
2.4.2	Estuarine zooplankton communities	28
2.4.3	Contrasting responses to environmental drivers	33
2.5	Discussion	35
2.5.1	Contrasting zooplankton communities and drivers.....	36
2.5.2	Differences in environmental conditions	37
2.5.3	Impacts of river regulation on environmental conditions	39
2.5.4	Management Recommendations.....	40
2.5.5	Conclusion	41
Chapter 3: Fine scale food web responses to a freshwater inflow event in a regulated		
Australian estuary		42
3.1	Abstract:	42
3.2	Introduction	42
3.3	Methods.....	44
3.3.1	Chemical and Chl <i>a</i> measurements.....	47
3.3.2	Bacteria, algal and zooplankton enumeration.....	47
3.3.3	Statistical analysis.....	48
3.4	Results	49
3.4.1	Discharge event and weir operation.....	49
3.4.2	Organic carbon and nutrient delivery	50
3.4.3	Bacterial and Algal Response	53
3.4.4	Zooplankton responses to discharge event	57
3.5	Discussion	60

3.5.1	Basal resources increase with flow	60
3.5.2	Mesozooplankton community responses	62
3.5.3	River Regulation and Management	64
3.5.4	Conclusion	65
Chapter 4: Inflow events drive resource use changes for mesozooplankton in a regulated estuary		
4.1	Abstract:	66
4.2	Introduction	66
4.3	Methods.....	68
4.3.1	Site Description.....	68
4.3.2	Physicochemical, DOC and chlorophyll <i>a</i> data	69
4.3.3	Stable Isotope Analysis.....	70
4.3.4	Stable Isotope Endmembers.....	71
4.3.5	Statistical Analyses	73
4.4	Results	75
4.5	Discussion	83
4.5.1	Allochthony following inflows	83
4.5.2	Allochthony and relationships to sPOM $\delta^{13}\text{C}$	85
4.5.3	Prolonged allochthony	87
4.5.4	Limitations	88
4.5.5	Conclusions.....	89
Chapter 5: Can zooplankton fed allochthonous carbon diets support and sustain juvenile fish?		
90		
5.1	Abstract	90
5.2	Introduction	91
5.3	Methods.....	93

5.3.1	Experimental Setup and Acclimation	93
5.3.2	<i>Artemia</i> and Feeding	94
5.3.3	Fish Sampling	96
5.3.4	POM and Tissue analyses	96
5.3.5	Statistical analyses	97
5.4	Results	98
5.5	Discussion	104
5.5.1	tDOM influence on growth	104
5.5.2	tDOM, inflows and recruitment	107
5.5.3	Limitations	109
5.5.4	Conclusion	110
Chapter 6: General Discussion and Conclusions		111
6.1	General Discussion	111
6.1.1	Planktonic responses in regulated systems	111
6.1.2	Role of terrestrial carbon	113
6.2	Further Research Directions	116
6.2.1	Temporal and spatial replication	116
6.2.2	Terrestrial carbon resources and co-metabolism	117
6.2.3	The role of mixotrophs	117
6.2.4	Responses of higher trophic levels to terrestrial inputs and heterotrophy	118
6.3	Management Recommendations	118
6.4	Conclusions	120
7.	References	121
8.	Appendices	155

List of Figures

Figure 1.1: A conceptual model of freshwater movement through an estuary. Freshwater enters the estuary from the upper catchment via rivers and tributaries and mixes with marine water to create a brackish environment before it is exported to the ocean. Variations in the magnitude of flows may cause flooding and lateral connections to the catchment, which may transport organic material and nutrient resources from catchment to the estuary. Image taken from OzCoasts 2015.	4
Figure 1.2: The algal grazer chain and microbial loop food webs. The grazer chain is a simpler and shorter food web, utilising primary producers as its basal resource, with zooplankton grazers passing energy onto higher trophic levels. The microbial loop may be subsidised by freshwater inputs of allochthonous dissolved organic carbon, transferring energy to higher order consumers through a combination of heterotrophic microbes, flagellates and ciliates. Energy transfers of autochthonous material are highlighted with green arrows, while allochthonous additions are indicated by black arrows.....	8
Figure 1.3: The position of the Manning and Williams estuaries (black dots) and their catchments (highlighted red) on the NSW coast.....	13
Figure 1.4: The Williams River transition from freshwater (left) to estuary (right) through Seaham Weir (centre) (Hunter Water 2011).	14
Figure 1.5: A view of the Seaham Weir gates from the wall. Freshwater (left) is prevented from entering the estuary (right). Photo courtesy of Prof. Simon Mitrovic.	14
Figure 2.1: Site and discharge gauge positions for the Manning River. Sites are denoted by black circles on the river channel. Site 1 is the most upstream point, with site numbers increasing downstream to Site 3 near Taree. WaterNSW gauge 208004 (Manning @ Killawarra) is indicated by the red square. Position of the study site along the NSW coast is indicated by the black circle in the inset image.	22
Figure 2.2: Site, weir and discharge gauge positions for the Williams River. Sites are denoted by black circles on the river channel. Site 1 is the most upstream point (near Seaham) with site numbers increasing downstream to Site 3 (closer to Raymond Terrace). Seaham Weir is denoted by the red triangle upstream of Seaham. WaterNSW gauge 210010 (Williams @ Glen Martin) is indicated by the red square upstream of Clarence Town. Position of the study site along the NSW coast is indicated by the black circle in the inset image.	22

Figure 2.3: Discharge (ML/Day – shaded grey area) of the Manning (A) and Williams (B) River estuary with salinity (ppt- points and lines) from the three sampling sites in each, Site 1 being most upstream and Site 3 the most downstream. Figures C and D show temperature (°C – thick grey line) and Chl a concentration (µg/L – points and lines) for the Manning and Williams estuaries respectively at the same three stations. Discharge data was taken from gauges 208004 (Manning @Killawarra) and 210010 (Williams @Glen Martin) as shown in Figure 2.1 and 2.2. Temperature is displayed as an average between the three sites of each estuary as there was little variation between sites. Chl a at Williams Site 2 in March 2018 was 82.5 µg/L.....	26
Figure 2.4: Comparisons of A) dissolved organic carbon (DOC (mg/L)), B) turbidity (NTU)), C) nitrates and nitrites (NO _x (mg/L)), D) filtered reactive phosphorous (FRP (mg/L)), E) Chlorophyll a (Chl a (µg/L)) and F) dissolved oxygen (DO (%)) concentrations between 30-day antecedent flow class (Q30 Class) conditions (low, moderate (Mod), fresh and flood) for the Manning (M) and Williams (W) estuaries. Error bars represent standard error of the mean. Number of samples taken within each flow class for Manning n = 41, 25, 10, 16 respectively and for Williams n = 29, 21, 18, 27.	28
Figure 2.5: Multidimensional scaling (MDS) ordination of the zooplankton community data for the Manning (M – open triangles) and Williams (W – closed triangles) estuaries. Ellipses represent 95% confidence interval. The stress of the model is indicated in the bottom right-hand corner.....	30
Figure 2.6: Density (Ind/L=individuals/L) of major zooplankton community groups (nauplii, copepodites and adults) for sites 1,2 and 3 in the Manning (A, C and E respectively) and the Williams (B, D and F) between January 2015 and June 2019. Other represents a combination of the remainder of the zooplankton from Table 2.1.	32
Figure 2.7: Distance-based redundancy analysis of the copepod communities of the Manning (A) and Williams (B) River estuaries for samples collected between January 2015 and June 2019. Species abbreviations in red correspond to those provided in Table 2.1. Environmental variable abbreviations are as follows: Q30 = 30-day antecedent discharge (ML/Day), Q60 = 60-day antecedent discharge (ML/Day), Temp = temperature (°C), NO _x = nitrate and nitrite concentrations (mg/L), FRP = filtered reactive phosphorous (mg/L), DOC = dissolved organic carbon (mg/L), Sal = salinity (ppt), DO = dissolved oxygen saturation (%), Turb = turbidity (NTU).....	34

Figure 3.1: Site, weir and discharge gauge positions for the Williams River. Study sites are denoted by black circles with Site 1 being the most upstream site, north of Clarence Town and Site 4 being the most downstream site, south of Seaham. Seaham Weir is denoted by the red triangle upstream of Seaham. WaterNSW gauge 210010 (Williams at Glen Martin) is indicated by the red square upstream of Clarence Town.	46
Figure 3.2: Discharge (ML/Day) for the Williams River and the salinity (ppt) and temperature (°C) of the two sites in the Williams River estuary between September 26 and December 12, 2018. Discharge was taken from NSW Water gauge 210010 - Glen Martin (grey shading). The sections of the hydrograph shaded black are the periods when the Seaham Weir gates were open (black) allowing flow downstream to sites 3 and 4.	50
Figure 3.3: Discharge (ML/Day) and concentrations of dissolved organic carbon (DOC), dissolved inorganic nitrogen (NO _x) and filtered reactive phosphorous (FRP) of 4 sites from the Williams River and estuary between the September 26 and December 12, 2018. Figures A, C and E were taken during the flow period and are insets of figures B, D and F. The dark bar on the x-axis of B, D and F indicates the period represented by A, C and E. Fine scale sampling during the inflow event was taken at sites Site 1 and 2, and Site 4, while post- flow samples were only taken at Site 3 and 4. Error bars represent standard error.	53
Figure 3.4: Discharge (ML/Day) and concentrations of bacterial cells (cells/L) in the Williams River estuary during (A) and following (B) the inflow event. Site 3 was not sampled intensively through the first flow peak. The dark bar on the x-axis of B indicates the period encompassing A. Samples for Site 3 on November 8 and 14 were compromised during analysis and hence not included. Error bars represent standard error of the mean.	54
Figure 3.5: Discharge (ML/Day) and, chlorophyll a (A, Chl a, µg/L) total algal biovolume (B, mm ³ /L) in the Williams River at Site 3 and 4 prior and following the inflow events. Figures C and D outline the algal community composition as a percentage of biovolume at Site 3 and 4, respectively. Genera comprising these 7 major groups can be found in Appendix 4.	56
Figure 3.6: Discharge (ML/Day) and abundances of nauplii (A), copepodite (B), adult copepod (C) and cladoceran (D) population concentrations (ind/L = individuals/L) across the October flow peaks. Site 2 was sampled with higher temporal resolution after the first peak to investigate fine scale changes in response to discharge. Error bars represent the standard error of the mean (SEM).	58
Figure 3.7: Distance-based redundancy analysis of the mesozooplankton community of the	

Williams River Estuary across an inflow event in 2018. “Pre-Flow” samples (red) are those taken on September 26, 2018, 10 days before the inflow event. “Flow” samples (green) are those from October 12 to 30, 2018, while weir gates were still being actively operated. “Post-Flow” samples (blue) are those from the November 2 to December 12, 2018, during which weir gates were almost exclusively closed. Significant environmental variables ($p < 0.05$) constraining the model are presented.....60

Figure 4.1: Site, weir and discharge gauge positions for the Williams River. Estuarine sites are denoted by black circles on the river channel. Site 1 is the most upstream black circle (near Seaham) with Site 2 being the downstream black circle nearer to Raymond Terrace. Seaham Weir is denoted by the red triangle upstream of Seaham. WaterNSW gauge 210010 (Williams at Glen Martin) is indicated by the red square upstream of Clarence Town. The red square between Site 1 and 2 is where the nutrient amendment experiment site.....69

Figure 4.2: A) Discharge (shaded peaks) and salinity, B) chlorophyll a (Chl a), C) dissolved organic carbon (DOC) D) turbidity and F) $\delta^{13}\text{C}$ of suspended particulate organic matter (sPOM) through the study period, January 2018 to August 2018, at Sites 1 and 2 in the Williams River estuary. Error bars represent the standard error of the mean.....77

Figure 4.3: Stable isotopic signatures of carbon ($\delta^{13}\text{C}$, ‰) and nitrogen ($\delta^{15}\text{N}$, ‰) for mesozooplankton consumers from the Williams River estuary tidal freshwater zone during pre- and post-flow periods between January and August 2018. June samples were assigned their own group “flowing” due to their temporal proximity to an inflow in June. Mean endmember source signatures (\pm SE) are also shown; Est. Alg = estuarine algae, Sed = sediment organic matter, Lom = *Lomandra longifolia*, Cas = *Casuarina glauca*.79

Figure 4.4: Mean percentage contributions of algae (estuarine algae) and terrestrial (*Lomandra longifolia*) food web resources to mesozooplankton diets between months where zooplankton $\delta^{13}\text{C}$ was enriched (January, March, May and June) and depleted (April, July and August) between January 2018 to August 2018 in the Williams River estuary as determined by Bayesian mixing model. Error bars represent standard error of the mean.....82

Figure 5.1: Bacterial cell concentrations (cells/ μL , $n=9$) for the 3 DOC addition treatments 0 mg/L, 5 mg/L and 10 mg/L in the *Artemia* on-growing chambers during the bio-encapsulation period. 0 hr measurements were taken minutes after the addition of leachate and *Artemia* to freshly prepared on-growing tanks. 40 hr measurements were taken from pre-prepared on-growing tanks to be used for feeding that day, approximately 40 hours after the

addition of leachate and Artemia. Error bars represent standard error of the mean (n =9).99

Figure 5.2: $\delta^{13}\text{C}$ (^{13}C) and $\delta^{15}\text{N}$ (^{15}N) stable isotope ratios for fish tissues at the beginning (Initial) and conclusion of the experiment for all 3 treatments (Fish 0 mg/L, Fish 5 mg/L and Fish 10 mg/L). Isotopic ratios are also displayed for particulate organic matter samples from the Artemia on-growing tanks (POM 0 mg/L, 5 mg/L and 10 mg/L) and Artemia (Artemia 0 mg/L, 5 mg/L and 10 mg/L) from the 3 treatments. Error bars represent standard error of the mean for each sample..... 100

Figure 5.3: Weight (A), standard length (B), fork length (C) and total length (D) of juvenile Australian bass for experimental carbon treatments of 0 mg/L, 5 mg/L and 10 mg/L across the study period. Measurements of fish when acquired are presented as “Initial” measurements. Error bars represent standard error of the mean (n = 30). Time points refer to T0 = start of experiment, T1 = 2 weeks, T2 = 4 weeks and T3 =6 weeks into the experiment. T3 was the final point of the experiment 101

Figure 5.4: Principal component analysis of truss-based morphology for juvenile Australian bass from treatment A (control – black circles), B (5 mg/L DOC addition – blue triangles) and C (10 mg/L DOC addition – red squares). PC1 was characterised by lengths of X2, X6 and X10 while PC2 represented X13, X8 and X12 (Appendix 13). X2 = length between the anterior tip of the upper snout and the origin of spinous dorsal fin, X6 = the length between the origin of spinous dorsal fin and the origin of pelvic fin, X10 = the length between the origin of spinous dorsal fin and the origin of anal fin, X13 = the length between the origin and insertion of the anal fin and X12 = the length between the origin of the anal fin and the insertion of the 2nd dorsal fin 103

Figure 5.5: Highly unsaturated fatty acid concentration of homogenised fish tissues for initial measurements and the control (0 mg/L), 5 mg/L and 10 mg/L treatments over the 6-week experimental period. A = docosahexaenoic acid (DHA, 22:6n-3), B = eicosapentaenoic acid (EPA, 20:5n-3) and C = arachidonic acid (ARA, 20:4n-6). Error bars represent standard error of the mean (n= 3). Time points refer to T0 = start of experiment, T1 = 2 weeks, T2 = 4 weeks and T3 =6 weeks into the experiment. T3 was the final point of the experiment..... 104

List of Tables

Table 1.1: Summary of the role of freshwater inflows in estuaries and the potential negative impacts of their reduction (adapted from Drinkwater and Frank 1994; Gillanders and Kingsford 2002 and Pierson et al., 2002).	6
Table 2.1: Mean, standard error and range for the density of most common zooplankton in the Manning and Williams estuaries between January 2015 and June 2019. Sample % represents the percentage of samples in which the zooplankton was found. Abbreviation in parentheses corresponds with usage in Figure 2.7.	29
Table 2.2: Results of multivariate abundance analysis (“mvabund” function) for factors River (n=2), Site (n= 3) and Flow Class (30-day antecedent flow class) n= 4) within the study. Q30 flow classes were determined using historic flow percentile curves. LRT = likelihood ratio test. Df = degrees of freedom.....	31
Table 2.3: Significant constraining variables in the Manning and Williams dbRDA models (Figure 2.7) as determined by Monte-Carlo permutational analysis (999 permutations).....	35
Table 4.1: Stable isotope ratios (‰) of organic matter sources for the Williams River estuary for carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) with standard deviation ($\pm\text{SD}$), and relevant C:N elemental ratios. The estuarine algal signature of $\delta^{13}\text{C}$ is derived from the treatment samples of the amendment experiment while $\delta^{15}\text{N}$ or estuarine algae is from the control samples of the amendment experiment, due to the effects of additional nutrients on the $\delta^{15}\text{N}$ of treatment samples.....	73
Table 4.2: Dominant mesozooplankton taxa in the Williams River estuary sorted by relative enrichment or depletion of $\delta^{13}\text{C}_Z$ through January to August of 2018, including where and when they were sampled, average $\delta^{13}\text{C}_Z$, $\delta^{13}\text{C}$ minus sPOM ($\delta^{13}\text{C}_Z - \delta^{13}\text{C}_{\text{POM}}$), average $\delta^{15}\text{N}$, $\delta^{15}\text{N}$ minus sPOM ($\delta^{15}\text{N}_Z - \delta^{15}\text{N}_{\text{POM}}$), expected TEF (trophic enrichment factor) range based on their trophic level (TL), and the primary, secondary and tertiary sources utilised by each group as identified by Bayesian mixing model (50% confidence interval). \pm symbols indicate standard error, GP = <i>Gladioferens pectinatus</i> , OS = <i>Oithona</i> spp., BM = <i>Bosmina meridionalis</i> , SC = <i>Sulcanus conflictus</i> , Est. Alg = estuarine algae, Sed = sediment organic matter, Lom = <i>Lomandra longifolia</i> and Cas = <i>Casuarina glauca</i>	81
Table 5.1: Two-way ANOVA results for bacterial concentrations in the bio-encapsulation tanks for treatments (0 mg/L, 5 mg/L, 10 mg/L) and bioencapsulation time (Bio-Time –	

0hours, 40 hours). Df = degrees of freedom, Sum Sq = sum of squares, Mean sq = mean of
squares.....98

List of Abbreviations

DOC	Dissolved organic carbon
FRP	Filtered reactive phosphorous
FWI	Freshwater inflow
OM	Organic Matter
NO _x	Nitrates and nitrites
NSW	New South Wales
Q30	30-day antecedent discharge
Q60	60-day antecedent discharge
tDOM	Terrestrial dissolved organic matter
tOM	Terrestrial organic matter
WSP	Water sharing plans

Ethics Statement

A study within this thesis involved the use of animals (Chapter 5). This research was approved by the University of Technology Sydney Animal Care and Ethics Committee under permit ETH19-3895. The research was conducted in accordance with the Australian Code for the Care and Use of Animals for Scientific Purposes (8th Edition 2013), the NSW Animal Research Act (1985) and Regulations (2010).

Abstract

Freshwater inflows are crucial to estuarine processes, regulating habitats and delivering important resources. However, river regulation has substantially reduced freshwater inflows to estuaries, affecting them negatively. This thesis aimed to enhance understanding of 1) how estuarine planktonic food webs responded to inflows in a regulated estuary; and 2) the importance of terrestrial carbon resources to estuarine food webs. These are important questions in estuarine flow-ecology and water resource management. To achieve this a suite of monitoring and manipulative studies were carried out in the Williams and Manning River estuaries.

A 4.5-year monitoring study comparing the unregulated Manning with the regulated Williams demonstrated the potential impacts of river regulation with results from the Williams contrasting strongly with the Manning. The Williams estuary was characterised by high concentrations of DOC and nutrient, regardless of flow conditions, with zooplankton communities consistently dominated by copepod nauplii, displaying no seasonal trends and responding positively to inflows and inputs of terrestrial DOC. Fine-scale monitoring of an inflow on the Williams estuary showed the importance of inflows in delivering terrestrial carbon and phosphorus resources and in stimulating bacterial and algal productivity. However, this did not translate to increased zooplankton production, relative to pre-flow periods.

Strong evidence was found for the importance of terrestrial carbon resources delivered to estuarine food webs by inflows. Analysis of zooplankton stable isotopes across a variable flow period showed that terrestrial carbon resources were being utilised by zooplankton following inflows, suggesting its importance in upper estuarine areas. Experimental results also indicated that terrestrial carbon in mesozooplankton food webs could contribute to the growth of juvenile fish of an endemic estuarine species. Together these results highlighted the importance and role of terrestrial carbon in estuarine food webs and productivity.

Individually my studies contribute new knowledge to important ecological and management questions in estuaries. Combined as a thesis they provide strong evidence for the importance of FWIs to estuaries and their food webs, highlighting the importance of terrestrial carbon as an energetic resource and contributor to estuarine productivity. This knowledge contributes to the wider literature recognising the importance of freshwater inflows to estuaries and supports their protection.