



MMCP Collaboration Final Report 2019

Prepared by: Daryl Nielsen, Gavin Rees, Paul Brown, Rick Stoffels, Paul McInerney and Rebecca Durant



Final Report: 2019

MDFRC Publication 217/2019



The MMCP Collaboration Final Report 2019

Final Report prepared for the Murray–Darling Basin Authority.

Murray–Darling Basin Authority Level 6, 33 Allara Street | GPO Box 1801 Canberra City ACT 2601

Ph: (02) 6279 0100; Fax: (02) 6248 8053

For further information contact:

Daryl Nielsen

PO Box 821 Wodonga VIC 3689 Ph: (02) 6024 9650

Email:daryl.nielsen@csiro.auEnquiries:cfe@latrobe.edu.au

Report Citation: Nielsen D, Rees G, Brown P, Stoffels R, McInerney P and Durant R (2019) MMCP Collaboration Final report 2019 –Report prepared for the Murray–Darling Basin Authority by The Centre for Freshwater Ecosystems, CFE Publication 217/2019, June, 61pp. doi.org/10.26181/5d19a18ad16c8

Finalised: July 2019

Cover Image: Ovens River, Victoria

Photographer: Ben Gawne

Acknowledgements: The La Trobe University offices are located on the land of the Latje Latje and Wiradjuri peoples. We undertake work throughout the Murray–Darling Basin and acknowledge the traditional owners of this land and water. We pay respect to Elders past, present and future.

This Project is supported through the Murray–Darling Basin Joint Governments. The Murray–Darling Basin Joint Governments are made up of;

- Department of Environment, Land, Water and Planning (Victoria)
- NSW Department of Primary Industries (New South Wales)
- South Australian Department for Environment and Water
- Department of Natural Resources and Mines (Queensland)
- ACT Environment and Sustainable Development (Australian Capital Territory)
- Department of Agriculture and Water Resources

Disclaimer:

© Murray–Darling Basin Authority for and on behalf of the Commonwealth of Australia

With the exception of the Commonwealth Coat of Arms, the Murray–Darling Basin Authority logo and The Murray–Darling Freshwater Research Centre logo, all material presented in this document is provided under a Creative Commons Attribution 3.0 Australia licence (http://creativecommons.org/licences/by/3.0/au/).

For the avoidance of any doubt, this licence only applies to the material set out in this document.



The details of the licence are available on the Creative Commons website (accessible using the links provided) as is the full legal code for the CC BY 3.0 AU licence (http://creativecommons.org/licenses/by/3.0/legalcode).

MDBA's preference is that this publication be attributed (and any material sourced from it) using the following:

Source: Licensed from the Murray–Darling Basin Authority under a Creative Commons Attribution 3.0 Australia Licence.

The contents of this publication do not purport to represent the position of the Commonwealth of Australia or the MDBA in any way and are presented for the purpose of informing and stimulating discussion for improved management of the Basin's natural resources.

To the extent permitted by law, the copyright holders (including its employees and consultants) exclude all liability to any person for any consequences, including but not limited to all losses, damages, costs, expenses and any other compensation, arising directly or indirectly from using this report (in part or in whole) and any information or material contained in it.

Contact us

Inquiries regarding the licence and any use of the document are welcome at:

Murray–Darling Basin Authority Level 6, 33 Allara Street Canberra ACT 2601 Email: copyright@mdba.gov.au

Document history and status

Version	Date Issued	Reviewed by	Approved by	Revision type
1	10/05/2019	Nathan Ning	Daryl Nielsen	Draft
1	30/05/2019	MDBA & JGR	Daryl Nielsen	Draft

Distribution of copies

Version	Quantity	Issued to
Draft	1 x word	Carla Tadich (MDBA) and JGR
Final	1 x PDF	MDBA and JGR

Filename and path:	Projects\MDBA\637 MDBA MDFRC Collaboration Agreement\Reports\Final report
Author(s):	Nielsen ¹ DN, Rees ¹ G, Brown ² P Stoffels ³ R, McInernery ⁴ P, and Durant ⁴ R.
Author affiliation(s):	¹ CSIRO Land and Water, Albury
	² La Trobe University, Mildura
	³ National Institute of Water & Atmospheric Research Ltd, Christchurch, NZ
	⁴ La Trobe University, Wodonga
Project Manager:	Daryl Nielsen
Client:	MDBA and the Joint Government Representatives
Project Title:	MMCP Collaboration
Document Version:	Final
Project Number:	M/BUS/637, 17/00796
Contract Number:	MD2881

Contents

Overview summary	1
Vegetation Dispersal	6
Response of basal resources to changing flows: how do small environmental flows influence stream structure and function at the lowest level	11
Understanding the ecological consequences of macroinvertebrate community-structure change	16
Fish Movement	19
Effects of river flows and temperature on the growth dynamics of Murray cod and golden perch	22
Basin Official Committee questions	23
Education	24
Communication	26
Appendix 1: Education Overview	23
Appendix 2: Communications Overview	23

Tables

Table 1. Specific objectives addressed by each of the research themes	1
Table 2. Potential risks to seed dispersal from key infrastructure in the MDB.	7
Table 3. Count of fish PIT tagged by species during training sessions with Lock 10 staff	21
Table 4. Summary of BOC questions, copies of reports are located on the La Trobe University's da23	ta depository.
Table 5. Summer cadetships undertaken as part of MMCP student funding	25
Table 6. Honours scholarships undertaken as part of MMCP student funding	25
Table 7. PhD Scholarships undertaken as part of MMCP student funding	26
Table 8. Water quality parameter values for each state.	

Figures

Figure 1. Relationship between the key monitoring and research programs: Long-Term Intervention Monitoring (LTIM), The Murray-Darling Basin Environmental Watering Knowledge and Research (MDB EWKR) and MMCP Collaboration (MMCP)
Figure 2. nMDS scaling of the community of seeds at each location. Blue – seeds in the drift in the river channel on the surface. Green – seeds drifting along the bottom of the river channel. Red – seeds passing into the wetland. Black oval indicates seed communities that are similar to each other
Figure 3. Percentage of seeds in the FP>90 category at each sampling time
Figure 4. Red gum blocks from the Wakool River (a) three weeks after deployment (half the block has been scrubbed of biofilm), (b) 11 weeks after deployment and (c) Mean Chlor-a concentration of biofilms grown in the three sites, from 24 October 2018–3 January 2019 (shaded area around lines represent 95% confidence intervals, boxplots representing upper and lower quartiles, whiskers 95 th percentiles)
Figure 5. Mean total mass (mg m ² top papel) and properties of total lipids (bottom papel) of occurric DUFAs

Figure 5. Mean total mass (mg m², top panel) and proportion of total lipids (bottom panel) of essential PUFAs alpha-linolenic (ALA; 18:3ω3), arachidonic acid ARA (20:4ω6) and linoleic acid (LA; 18:2ω6) within

- Figure 9. Response of Murray cod growth to river flows and temperature visual summary. Examples from the Murrumbidgee River are presented, but these examples are representative of the MDB as a whole. (a)–(c): Observed annual temperature and discharge time series, as well as the growth time series predicted by the final model (± 95% confidence interval) for different life-stages of Murray cod. Vertical dashed lines in (a)–(c) denote La Nina floods and predictions of growth without confidence intervals denote predictions beyond domain of covariates for this river. (d)–(e): predicted growth response to annual discharge (flow percentiles; e.g., Q50 is the median) and annual temperature (°C), respectively, of different life stages. Boxes present the uncertainty in the effect of a covariate (one of annual discharge or temperature) generated by the interaction that covariate has with temporal variation in the other covariate (see CFE Publication 222/2019).25

- Figure 12. Photograph of mine tailings overlying original floodplain surface (marked by red line; Loddon River). 34
- Figure 13. Arsenic (top) and loss of ignition (bottom) profiles for the Loddon River sites. Profiles extend through the overlying tailings through the original (relic) floodplain (boundary marked by 'contact).
 Figure 14. Speciation diagram for the system Zn²⁺ + H⁺ + Cl⁻ + CO₃²⁻ + SO₄²⁻ in a simulated freshwater medium

-		-				
	(Powe	ll et al. 2015).	 	 	4	0

Overview summary

MMCP Collaboration (MMCP) contributed to the commitments the Murray–Darling Basin Authority (MDBA), La Trobe University (LTU) and CSIRO, have to the generation and adoption of freshwater ecological knowledge through collaboration, through a five year investment (2014-2019) project. The Murray–Darling Basin Joint Governments, MDBA and LTU have worked together to maintain research capability and contribute supporting science to underpin the Basin-Wide Watering Strategy (BWS: Table 1).

 Table 1. Specific objectives addressed by each of the research themes.

Ve dis	egetation spersal	Response of basal resources to changing flows	Consequences of macroinvertebrate community- structure change	Fish movement	Impact of hydrology and climate on fish growth
1.	Improving unde biodiversity	rstanding of the rela	itionship between flow	r, ecosystem funct	ion and
	\checkmark		\checkmark		
2.	Improving mana and complemer	agers' capacity to prontary natural resource	edict the environmenta ce management	al outcomes of wa	ter management
			\checkmark		
3.	Improved capac water managen	ity to evaluate the t nent and climate sce	hreats to ecosystem fu narios	nction and divers	ity under a range of
	\checkmark				
4.	Improving capa effectively and	city to assess ecosys efficiently achieve er	tem condition and ider nvironmental objective	ntify the intervent s	ions most likely to
	\checkmark	\checkmark			

MMCP directly addresses the following three Basin Plan environmental objectives:

Protect and restore water-dependant ecosystems.

• The maintenance of connectivity both longitudinally and laterally is recognised as being important in the protection and restoration of aquatic ecosystems. The MMCP aims to provide managers with the tools to make informed decisions on (i) how effectively the creation of longitudinal connectivity from the Sea-to-Hume has restored native fish communities, and (ii) how the operation of infrastructure (pumps/regulators) to restore lateral connectivity between rivers and wetlands will lead to changes in vegetation communities by selecting either for or against seeds with specific traits.

Protect and restore the ecosystem functions of water-dependant ecosystems.

 The management and restoration of native fish populations has primarily targeted the maintenance of flows and habitats that promote recruitment and growth of larval and juvenile fish. The success of these actions may be limited due to a poor understanding of (i) what appropriate flow regimes are required to support the growth of native fish, and (ii) whether the appropriate food resources occur under current conditions to support the recruitment and growth of native fish. The MMCP will provide water resource managers with the knowledge on how to manipulate flow regimes to support and maintain food resources and promote the growth of native fish.

Ensure that water-dependant ecosystems are resilient to climate change and other risks and threats.

• The MMCP will provide water resource managers with the knowledge on how best to manipulate water regimes to maintain ecosystem function (including native seed dispersal and growth of aquatic organisms) and protect water-dependant ecosystems as the demand for water resources increases under climate change scenarios.

To sustain the collaboration, MMCP delivered on five research projects, responded to questions agreed to by the Basin Officials Committee in the form of synthesis papers (two papers per year) and through a capacity building program of supporting post graduate students through scholarship funding.

The MMCP research program is based around five themes:

- Vegetation dispersal.
- Fish movement.
- Fish population and community dynamic modelling.
- Linking macroinvertebrate community structural changes to ecosystem outcomes.
- Response of basal resources to changing flows.

Relationships between MMCP, LTIM and MDB EWKR research programs

La Trobe University undertakes a range of research and monitoring programs throughout the Murray–Darling Basin. As well as the MMCP, the Centre is currently coordinating two complementary large-scale programs, the Murray–Darling Basin Environmental Water Knowledge and Research Project (MDB EWKR) and Commonwealth Environmental Water Office's (CEWO) Long-Term Intervention Monitoring (LTIM).

The MMCP complements these programs. All three programs monitor or undertake research into the ecology of rivers in the Murray–Darling Basin (MDB). Critically, where there are overlaps in research themes (e.g. fish ecology), each program investigates different aspects of the issue. In addition, the MMCP program has an emphasis on synthesis of existing data and building research capacity within freshwater ecology through the sponsoring of PhD 'top-up' scholarships.

The Commonwealth Environmental Water Office's Long-Term Intervention Monitoring (LTIM) program

The LTIM program monitors the outcomes of environmental flow interventions (water actions) as part of the monitoring and evaluation phases of the CEWO adaptive management framework. The primary objective of the LTIM project is to monitor the outcomes of environmental flows and support evaluation of their contribution to the achievement of Basin Plan objectives. The project monitors six broad indicators (hydrology, ecosystem diversity, river metabolism and water quality, vegetation, fish and generic diversity) at seven selected areas. The outcomes are evaluated at the 'area' scale and an evaluation is also undertaken at unmonitored sites using a multiple lines of evidence approach. The outputs of these evaluations are then integrated to provide an evaluation at the Basin scale. The program is focussed on monitoring outcomes that are directly relevant to Basin Plan environmental objectives such as biodiversity (e.g. ecosystems, fish) and ecosystem function (e.g. hydrological connectivity, river metabolism) (Figure 1). What the program does not do, is examine the processes that link changes in flow to changes in the indicator. For example, fish population changes may be due to changes in habitat, connectivity, food or predation, but these process lie outside the scope of LTIM. The LTIM program also does not consider the influence of non-flow drivers on the outcomes of water actions, as these processes are not monitored.

The Murray–Darling Basin Environmental Water Knowledge and Research (MDB EWKR) project

The objective of the MDB EWKR project is to 'improve our understanding of the influence of flow on the condition of the Basin's water-dependent ecosystems'. It seeks to enhance environmental water management and thereby improve environmental outcomes through the generation of new knowledge that improves our understanding of both flow responses, and the way that non-flow drivers (stressors) may affect the outcomes of environmental flows. The MDB EWKR builds on the assessment and monitoring projects being undertaken by management agencies with benefits to both managers and researchers. The MDB EWKR project will identify activities that will lead to improvement in managers' capacity to predict the outcomes of environmental flows. Wherever possible, these activities will seek to build on previous work (data analysis) or complement other activities occurring throughout the Basin, including Basin Plan Monitoring and Evaluations and LTIM. It is anticipated that the project's outputs will be useful in interpreting the assessments undertaken by Basin Plan Monitoring and Evaluation, support improved planning and operational decisions around environmental flows and inform the evaluation of intervention monitoring data; helping to identify refinements to the prioritisation and design of environmental flows.

Project alignments

The LTIM program has quite specific objectives and questions that are appropriate to its role in informing adaptive management. The LTIM data will contribute to an assessment of condition across the Basin.

The MDB EWKR project has a much broader brief, but its relationship to the LTIM is important in both improving the outputs of the MDB EWKR project and the application of the project's outputs to key steps in the adaptive management framework, condition assessment and evaluating the outcomes of interventions.

The MMCP seeks to generate new knowledge by undertaking targeted research on specific topics that have been identified as key knowledge gaps by LTU, MDBA and the State funding jurisdictions. Outcomes from the five research themes and responses to the synthesis questions will complement activities undertaken under MDB EWKR. Additionally, the response to the synthesis questions will provide guidance to water resource managers about the effectiveness of current water resource management strategies within the Basin, which will guide the ongoing adaptive management of water resources within the Basin.

Together these major research projects provide a complementary suite of activities that will support management of the MDB into the future. Specific benefits to management include the following:

• Improved understanding of ecosystem flow requirements and the factors that facilitate or limit outcomes will enable more effective and efficient use of environmental water.

- Improved predictive capacity of the outcomes of environmental flows will support planning and operational decisions around environmental flows. This will be particularly important in situations where volumes of water are less then specified water requirements, for example during periods of drought.
- Increased understanding of the influence of other stressors may lead to the development of innovative and efficient restoration initiatives. This will be particularly important in systems with limited volumes of environmental water, such as some rivers in the northern Basin.
- Identification of complementary natural resource management options may enhance outcomes of environmental flows. These will both enhance efficiency of environmental flows and provide a focus for engagement with regional communities.



Figure 1. Relationship between the key monitoring and research programs: Long-Term Intervention Monitoring (LTIM), The Murray-Darling Basin Environmental Watering Knowledge and Research (MDB EWKR) and MMCP Collaboration (MMCP).

Vegetation Dispersal¹

Key recommendations/options

Findings from these results indicate that the modification of flow paths by the use of infrastructure is likely to influences the dispersal patterns of seeds. This has the potential to influence the communities within wetlands as they recover from disturbances such as drying. How many and which species are able to be transported into these habitats will depend on the type of connection and life history traits of the available species pool.

To maximise the potential for seeds to be transported into wetlands, consideration needs to be given to the type of delivery structure being implemented and how that infrastructure is operated (Table 2).

¹ For more detailed information on this project refer to <u>CFE Publication 218/2019</u>.

Table 2. Potential risks to seed dispersal from key infrastructure in the MDB.

Consideration			Best likely
Structure	Impact	Mitigation	outcome (rank)
 <u>Unregulated channel</u> Connection between river and wetland unimpeded by any structure. 	• Nil.	• Nil.	<u>1 (best)</u>
 Pumps Connection between river and wetland maintained by pumping water. Water pumped sub- surface. 	 Selects for seeds floating sub-surface. Potential for seeds to be damaged. 	 Adjust height of float value to modify depth from which water is pumped. 	<u>5</u>
 <u>Undershot (sluice)</u> <u>regulators</u> Flows modified by raising sluice and allowing water to flow underneath. Used to prevent water moving either into or out of a wetland. 	• Entrapment of seed and other debris drifting on the surface.	Complete opening of sluice.	<u>4</u>
 <u>Overshot (drop-board)</u> regulators Flows modified by removing or adding boards. Used to prevent water moving either into or out of a wetland. 	 May reduce the potential for seed dispersal due to reduced flows and potential entrapment of seeds. 	 Removal of all boards to allow maximal water movement into wetlands. 	<u>3</u>
 <u>Tilting (Lay) flat gates</u> Flows modified by tilting weir on its bottom horizontal axis. Used to prevent water moving either into or out of a wetland. 	 Minimal. Titling of gates should allow seeds to be washed in to wetlands. 	 Full tilt of weir(s) for maximum water movement into wetlands. 	<u>2</u>

Background

The movement of propagules within the landscape is an important factor in maintaining the diversity of aquatic and riparian plant communities. Changes in flow regimes, or hydrological connectivity, are likely to significantly impact the distribution of aquatic and riparian plants. Changed connectivity may occur through the disconnection of components of the landscape (i.e. between rivers and wetlands) caused by changes in flow regime, and the construction of barriers that physically impede dispersal. Research globally has indicated that provision of pathways for the dispersal of seeds and propagules is important in the restoration and rehabilitation of wetlands. The restoration and maintenance of lateral connectivity in achieving ecological outcomes has been recognised in the MDBA's Basin-Wide Environmental Watering Strategy (BWS) to achieve ecological outcomes for plants as well as birds and fish.

Information from this project will provide water resource managers with the knowledge on how best to manipulate water regimes to maintain ecosystem functions (including native seed dispersal) and protect water dependant ecosystems as the demand for water resources increases under climate change scenarios.

The objectives of this research project are to:

- Understand the spatial and temporal movement patterns of wetland and riparian vegetation.
- Describe the physical characteristics of seeds that will facilitate dispersal.

Summary

The distribution and abundance of aquatic and riparian plants is strongly influenced by hydrology and the availability of water. Changes in flow regimes or hydrological connectivity, are therefore likely to significantly impact the distribution of aquatic and riparian plants. Changed connectivity may occur through the disconnection of components of the landscape caused by changes in flow regime, construction of barriers that physically impede dispersal or through the artificial movement of water between rivers and wetlands. River regulation has altered hydrological connectivity and flow regime characteristics throughout the MDB. Water control measures are increasingly being used to manipulate flow within the system to meet ecological needs; however, the use of infrastructure may led to a loss of ecological integrity by reducing the movement of biota and other associated material. These alterations are likely to have affected the dispersal patterns of many plant species.

This project seeks to improve our understanding of the movement of seeds through pumps and regulators. In this study, we test four hypotheses. We hypothesise that:

- 1. The seed communities drifting on the river surface differ to those drifting along the bottom of the river. This is important as the manner in which water is moved into wetlands has the potential to select either for seeds drifting on the surface or seeds drifting sub-surface.
- 2. Seeds moving into wetlands un-impeded by any structures will reflect seed communities drifting on the surface of the river channel.
- 3. The seed community that passes through pumps will reflect the seed community drifting sub-surface in the river channel.
- 4. Seeds moving into wetlands through regulators will reflect seed communities drifting on the surface of the river channel.

Results from this research indicate that the seed communities drifting on the surface of rivers differ from those that that drift sub-surface. This means that the different types of infrastructure used in the MDB are likely to select for different communities (Figure 2).

- Pumps will select for seeds drifting sub-surface. This may be offset if pumps were fitted with floating foot values that allowed water to be taken from the surface.
- Partially opened undershot regulators will select a community of seeds that is likely to be a subset of the community floating on the surface of rivers. It is only a subset because when partially opened an accumulation of debris may entrap seeds on the surface. This would be offset if regulators were fully opened.
- Although not tested, when overshot regulators are installed they should be fully opened where possible to minimise any effect of seed entrapment.



Figure 2. nMDS scaling of the community of seeds at each location. Blue – seeds in the drift in the river channel on the surface. Green – seeds drifting along the bottom of the river channel. Red – seeds passing into the wetland. Black oval indicates seed communities that are similar to each other.

Seeds from 59 species were tested for their ability to float and a number of physical parameters were measured. Each species was classified into one of six percentage groups based on its seed's ability to float. These included FP>90 (91-100), FP90 (76-90), FP75 (51-75), FP50 (26-50) and FP25 (11-25) FP10 (0-10), where FP>90 indicated that the majority of seeds floated and FP10 indicated that the majority of seeds had sunk.

- No physical feature measured could be related to a seed's ability to float.
- Exotic species were not more likely to float than native species.
- There does not appear to be a difference in the floatation of seeds from broad functional groups (aquatic, emergent, terrestrial).

The majority of seeds did not float on the surface for extended periods of time and after 24 hours 50% of seeds had sunk (Figure 3). However, under flow regime of the Murray River, a seed could potentially float for several kilometres in that period.



Figure 3. Percentage of seeds in the FP>90 category at each sampling time.

Findings from this research indicate that:

- Different types of infrastructure will influence the transport of seeds and other propagules into wetlands.
- Seeds drifting on the surface are likely to be transported short distances before they either become entrapped or sink.
- Seeds drifting sub-surface may potentially be able to be transported longitudinally over much greater distances.
- The floating ability of introduced species did not differ from native species.

Strategic alignment

This project complements activities undertaken with other large projects within the MDB that are aimed at understanding plant responses to environmental watering of floodplains and wetlands (MDB EWKR, LTIM and The Living Murray Project (TLM)). Specifically this project seeks to provide possible explanations on why the use of infrastructure to manipulate environmental watering events, to benefit floodplain and wetland plants may not meet the desired objectives of the watering event.

Journal Outputs

Manuscripts planned for submission:

- How does infrastructure influence the dispersal of riparian and wetland plants on to floodplains?
- Do morphological characteristics influence seed dispersal capacity?

Response of basal resources to changing flows: how do small environmental flows influence stream structure and function at the lowest level²

Key recommendations/options

Aspect	Findings	Recommendations
Dissolved organic carbon	 Protein-like DOC in the water column is linked to bacterial production in place of algal activity. Allochthonous DOC important. 	 Protect allochthonous sources of DOC (e.g. riparian vegetation, inundation of floodplains) within lowland rivers. Where possible, inundate banks and floodplains to provide allochthonous carbon into the river channel.
Biofilms	 Biofilms responding to environmental drivers. Inundation duration of up to 11 weeks provides high quality biofilms. 	 Consideration of flow duration to optimise biofilm quality. Submersion of surfaces for 5 weeks yielded peaks in some key measures of biofilm quality. Some further improvement in biofilm quality up to 11 weeks after initial submersion.
Stream metabolism	 Surfaces are heterotrophic and consume more oxygen than they produce via microbial processing of organic material. Most production in Edward-Wakool Rivers is occurring in the water column. 	 Systems shown to be net consumers of oxygen under steady high flows. Recommend further research under various environmental flow scenarios to gauge ecosystem response to ultimately optimise flow management.

Background

Initially, the focus of this project was to assess changes in biofilm quality in response to managed flows in rivers within the Edward–Wakool River system that had environmental flows planned for 2018–2019. However, due to extremely dry conditions throughout the MDB in 2018, all planned environmental flows for the catchment were suspended in order for the MDBA to transfer water to Lake Victoria. While changes to the planned environmental flow regime were disappointing, it provided us with a unique opportunity to investigate successional patterns of biofilms through time, replicated in three adjacent streams under relatively constant flow conditions. Information surrounding basal food quality under such scenarios is of particular applied interest, since a better understanding of how biofilm quality and ecosystem function changes through time could help inform decision making by water managers for optimisation of flow duration.

² For more detailed information on this project refer to <u>CFE Publication 219/2019</u>.



Figure 4. Red gum blocks from the Wakool River (a) three weeks after deployment (half the block has been scrubbed of biofilm), (b) 11 weeks after deployment and (c) Mean Chlor-*a* concentration of biofilms grown in the three sites, from 24 October 2018–3 January 2019 (shaded area around lines represent 95% confidence intervals, boxplots representing upper and lower quartiles, whiskers 95th percentiles).

Summary

Fluorescence excitation emission scans of dissolved organic matter in the Neimur River, Wakool River and Yallakool Creek water followed by PARAFAC analysis revealed the presence of three main dissolved organic carbon (DOC) components. Our work showed the presence of a heterotrophic microbial community within the water column. The lack of a significant difference between water incubated under light and dark conditions and the production of protein-like DOC within all three rivers also suggests that protein-like DOC in the water column is linked to bacterial production in place of algal activity. The high abundance of the humic-like component found within these systems is indicative of the important role allochthonous DOC plays in these systems. Protection of allochthonous sources of DOC (e.g. riparian vegetation, inundation of floodplains) is an important management consideration.

It took approximately five weeks for chlorophyll- *a* in biofilm to increase markedly (Figure 4), indicating algal growth. We show a range of key metrics to which biofilms are responding (e.g. discharge, nutrients, turbidity). This response coincided with increases in some essential polyunsaturated fatty acids (PUFA) critical for animal development (e.g. arachidonic acid ARA (20:4 ω 6), Figure 5). Green algae is known to be an excellent source of PUFA, and here we show that some essential fatty acids peaked in concentration after 11 weeks deployment. This was supported by our biofilm Carbon:Nitrogen (C:N) values, which were lowest after 11 weeks (lower is reflective of higher food quality). This is important information, since there is evidence in the literature that biofilms become a poorer quality food source through time as they become dominated by filamentous algae and cyanobacteria. Here we show that biofilm quality was retained for up to 11 weeks following inundation, and based on some key metrics (e.g. C:N, fatty acids profiles), peak quality was reached at the end of the study. More work is required to extend our study past 11 weeks, but the information generated from this research has important implications for directing inundation duration of managed flows.



Figure 5. Mean total mass (mg m², top panel) and proportion of total lipids (bottom panel) of essential PUFAs alpha-linolenic (ALA; 18:3 ω 3), arachidonic acid ARA (20:4 ω 6) and linoleic acid (LA; 18:2 ω 6) within biofilms grown in the three study sites, from 24 October 2018–3 January 2019 (shaded area around lines represent 95% confidence intervals, boxplots representing upper and lower quartiles, whiskers 95th percentiles).

Our work indicates that during steady high flows over spring and summer that streams within the Edward-Wakool River system are strongly heterotrophic (Figure 6). Importantly, our technique showed that gross primary production (GPP) in these ecosystems is predominantly generated within the water column by phytoplankton. Surfaces (combining hard surface biofilms on snags and soft benthos) are predominantly heterotrophic and consume more oxygen than they produce via microbial processing of organic material. Our data provides important baseline information of ecosystem dynamics within these systems that will be useful for comparison with metabolism responses to managed flows.



Figure 6. Stream metabolism dynamics GPP (A), NPP (B), CR (C), and littoral zone NPP (D) estimated as $O_2 mg/L$ per hour calculated from open water estimates (crosses) and light and dark bottles (circles) from the Niemur River, Yallakool Creek and Wakool River from 24 October 2018–3 January 2019 (error bars ±1 SE, shaded area around lines represent 95% confidence intervals).

Strategic alignment

Knowledge generated by this project directly aligns with other large projects within the MDB that are interested in understanding which environmental flow regimes can best support the food webs necessary for successful breeding and independent survival of waterbirds and fish (e.g. MDB EWKR, LTIM). Our approach to understanding basal resources quality within food webs is directly in alignments with the MDB EWKR food web theme while information generated from our assay approach to estimating pelagic production aligns with work conducted under the LTIM project.

Journal outputs

Manuscripts planned for submission:

- Patterns in benthic algal fatty acid compounds in lowland rivers.
- Successional patterns of biofilms in lowland rivers following inundation.
- Dissolved organic matter varies temporally within the Neimur, Wakool and Yallakool Rivers.

MMCP Collaboration Final Report 2019

Understanding the ecological consequences of macroinvertebrate community-structure change³

Key recommendations/options

Macroinvertebrate community response to flow

- There was no relationship between flow variables and nutritional status of prey (measured as C:N ratio), or species richness.
- Flow only explain a very small part (<10%) of the variation in macroinvertebrate biomass (effectively amount of energy available to consumers).
- These results suggest that a combination of factors are driving the biomass, with other limiting factors playing a clearer role in driving abundance. Future work is required to test alternate modelling techniques.

Taken in isolation, macroinvertebrate abundance measured at the scale of this component can not necessarily be predicted for a series of flow variables. Combination with other factors that are likely to promote increased abundance, for example woody debris. The latter complimentary measure needs to be carried out in conjunction with flow modifications, and the optimal conditions resulting in increased food availability.

• There were improvements in the abundance of macroinvertebrates in the Murray River between 1980 and 2013. However, the biomass and diversity of macroinvertebrates appeared to decline in 2014 around Yarrawonga and Lock 9. Further investigation is required to identify potential reasons for this decline.

Yabby nutritional ecology

- Yabbies require a diverse range of food sources to maintain highest growth rates and poor quality diets result in a small but significant change in their nutritional value as prey for fish.
- While essential dietary fatty acids could be derived from food sources in rivers and wetlands, rivers and wetlands differed in their ability to supply the essential fatty acids. Thus, an optimum balance of essential fatty acids would be achieved for yabbies if they were presented with both wetland and river conditions.

These data provide evidence based information on the value of eflows. They also provide a valuable basis for modifying any continued monitoring, particularly with the development of complimentary measures in river system management.

Background

The initial aims of this theme were to: 1) build on the understanding of macroinvertebrate species richness and total biomass that could be derived from the long-term monitoring program of macroinvertebrates in the Murray River, and 2) advance the understanding and application of knowledge on nutritional ecology in freshwater systems, to predict better outcomes from environmental water.

The 35-year monitoring program of macroinvertebrates in the Murray River (currently the River Murray Biological Monitoring Project (RMBMP)) provided an extensive dataset that could potentially improve our knowledge of the functional role of macroinvertebrates in rivers more generally. Food quantity and quality are just two elements that can be important in determining community

³ For more detailed information on this project refer to <u>CFE Publication 220/2019</u>.

structures, thus influencing top predators such as fish. Since macroinvertebrates represent one form of prey item for consumers, a change in the type of prey items, due to the change in macroinvertebrates, could mean: 1) that the overall energy available for consumers may have altered, and/or 2) the nutritional landscape for consumers also changes.

Yabbies (*Cherax destructor*), along with other decapods, are a major source of food for fish. Yabbies are omnivorous, deriving food from a range of detrital and protein food sources, making them an important linking part of the riverine food web. Changes can occur in the C:N ratio of yabbies in response to different riparian vegetation, so any environmental flow that can mediate a changed environment could likely lead to similar changes in the nutritional value of yabbies, leading to a change in their nutritional value.

To address the key background issues, three overarching questions were addressed by this component of work:

Can flow variables be used to predict the quantity and quality of food resources in rivers?

Do primary food resources alter the growth rates and nutritional quality of key fish prey?

Does floodplain connectivity lead to any improved nutritional quality of key fish prey?

Summary

An analysis of 30 years of data on the abundance and species richness of benthic invertebrates in the Murray River, showed abundance increased across all sites after the 1993 flood and declined after peaking during the millennium drought. Richness was more variable with respect to sites.

Our modelling showed no relationship between species richness, the overall nutritional value (measured as C:N ratio) and flow. Log-normalised flow parameters could only explain less than 10% of the variation in macroinvertebrate abundance, thus flow alone isn't a limiting factor, and our results suggest that other factors are contributing to limited growth. We suggest, for example, that insufficient surfaces are available for colonisation at a scale that would see sufficient increases in macroinvertebrate biomass.

Diet had a profound effect on the growth of yabbies; food with a very high C:N ratio (38) provided nourishment for yabbies, but they barely increased their body mass over the entire experiment. Significantly faster growth occurred on bloodworms (C:N ratio = 4.6), but not as great as growth on commercial pellets (C:N ratio = 6.8), showing that C:N ratio alone was not an ideal predictor of growth. While food sources at river and floodplain sites provided adequate nourishment for yabbies, their resources were sufficiently different, leading to yabbies themselves having different nutritional values, depending on their origin.



Figure 7. The long-term response of benthic macroinvertebrate biomass and species richness to discharge. The top row shows average flows from 1980 to 2013. The blue line shows when flow was above average and the orange line shows below average flow. Ovals highlight the millennium drought. The middle row shows community biomass response over time and the bottom row shows taxa richness.

Strategic alignment

This project has very strong links to the MDB EWKR food webs project, which described patterns of basal energy flow among river channel, wetlands and anabranches. Both projects focussed their efforts on the Ovens River floodplain, providing an additional strong link. Our work concentrated effort on a major species that forms prey for fish, thus building on MDB EWKR.

This project also links very strongly with the component of the MMCP; Biofilm succession patterns and ecosystem dynamics in the Edward-Wakool River system.

There is also now a wide body of research in Australia that is examining the nutritional importance of riverine food resources. This aspect of freshwater ecology continues to attract major interest.

Journal outputs

Manuscripts planned for submission:

• Habitat diversity leads to higher nutritional value of a key prey item for fish, a nutritional ecology of yabbies.

Fish Movement⁴

Key recommendations/options

Murray River weirs are being operated in an increasingly dynamic way to achieve environmental outcomes. Weir pool manipulation and environmental operations present unique challenges for the operation of fishways. Passive Integrated Transponder (PIT) tag readers provide valuable data on the passage of fish which can inform the operation of the fishway, and ultimately result in improved management of flows to optimise fish passage. This dataset has also proven useful for stakeholder engagement, reporting fishway movements, and scientific research. As PIT tag readers provide useful information on the performance of fishways they in turn reduce the need to trap fish for fishway assessment, an exercise that is labour intensive and taxing on fish.

The benefits of continuing to train and having trained PIT tagging staff can aid in the following outcomes:

- Improved knowledge of the efficiency of fish passage at Murray River fishways and how fish utilise the fishways.
- Assessment of fishway performance where weir pools are operated outside normal levels (e.g. during weir pool raising and lowering events).
- Reporting of PIT tag data as a means of engaging fishway operators and other relevant stakeholders.
- Estimate of the rate of ascent success for Murray River fishways, based on the movement of fish within fishways subsequently encountered by tagged fish.
- Use of PIT tag data to improve fishway function and optimise operations.
- Improved understanding of upstream and downstream fish movements and seasonal patterns of migration to inform operations.

The improved data set produced, as a result of the tagging program, may also contribute to additional scientific research and knowledge including:

- Understanding of spatial behaviour of freshwater fish over both a small spatial scale (within fishways) and a large spatial scale (between fishways).
- Specific knowledge of the migratory pathways of native fish and the ecological benefit of the fishways.
- Behaviour of native fish, migratory pathways, recruitment sites and preferred habitats of native fish populations.
- Linking with other fish community-condition data sets to increase our understanding of the role of fish movement in rehabilitation of river-ecology.

Background

The Sea to Hume fishway program saw the construction of fishways at 12 locks and weirs and five barrages along the main stem of the Murray River from 2001 to 2014, opening up over 2,000 km of waterway for fish passage. The Murray River Fishways Assessment Program (MRFAP) was established to monitor the effectiveness of the fishways following construction. MRFAP

⁴ For more detailed information on this project refer to <u>CFE Publication 221/2019</u>.

demonstrated that the fishways on the Murray River effectively passed diverse and abundant fish, comprising a wide range of size classes.

The MDBA's *Native fish Strategy* (NFS) and the Sea to Hume fish passage restoration program within it, was a fish-population rehabilitation program on a grand-scale. Originally, a 50-year strategy, the NFS was superseded in 2014 by the more holistic, Basin-Wide Environmental Watering Strategy (BWS).

The NFS followed by the BWS form probably the largest native fish-community rehabilitation plan in Australia. Fish movement is an essential behavioural component of many species within the MDB native fish communities. Expected outcomes of the BWS around fish movement include targets for fish passage.

Fish passage is presently monitored by manual trap-count-and-release activities, and by an efficient automated PIT tagged fish monitoring system at fishways. Without continued PIT tagging of fish, there may soon be insufficient tagged-fish in the MDB to effectively use automated monitoring to evaluate the expected outcomes of the BWS. There is presently a large (>6 million data points and growing) dataset of fish detections at fishways, which has the potential to increase our understanding of fish movement behaviour to facilitate improved ecological outcomes from water management.

Summary

Since the conclusion of the NFS in 2014, numbers of tagged fish in the Murray River have declined below those necessary to produce meaningful data from PIT tag readers installed on fishways. In an effort to increase the number of PIT tagged fish in the river (and potentially using the fishway), training was provided to lock staff in the anaesthesia, tagging, handling and care of fish caught in fishway traps.

Training of WaterNSW staff at Lock 10 was undertaken with an adequate level of competency reached and preliminary training has been undertaken with Lock 15 staff. These skills need to be consolidated at their 'home' lock to complete their training. Training was structured around practical tagging followed by data-entry and uploading to the *FishNet* database (<u>https://PIT-tags.com.au/FishNet/</u>); following procedures in the training course developed earlier in the project, 'Lock keeper's Guide to PIT Tagging Fish at Lock 10'.

Training has concentrated on developing safe practices and competency in tagging fish rather than achieving any objective count of tagged fish. However, for completeness, the numbers of fish tagged during training are presented by species and year in Table 3.

Fish tagged	2015	2016	2017	Species totals
Silver perch	11	5	1	17
Carp	35			35
Golden perch	134	64	90	288
Murray cod	1			1
Bony herring	1			1
Annual totals	182	69	91	342

Table 3. Count of fish PIT tagged by species during training sessions with Lock 10 staff.

Strategic alignment

The BWS is an important part of the implementation of the Basin Plan and in addition to fish, considers outcomes for waterbirds, vegetation and hydrology. From a MDB fish perspective, both the NFS and BWS combine to form what is essentially a basin-scale fish-population rehabilitation program for the recovery of native fish communities over a 20-year period spanning 2004–2024.

The Fish Movement theme within the MMCP has a single objective in, building the capacity of staff trained to PIT tag fish. This objective arises from a need to maintain adequate numbers of PIT tagged fish within the Murray River to enable the automated logging of fish passage by a system of PIT detectors at fishways.

Publication Outputs

Huntley S, Brown P (2016) Lock Keeper's guide to PIT tagging fish at Lock 10. Report prepared for the Murray–Darling Basin Authority by The Murray–Darling Freshwater Research Centre. MDFRC Publication 112/2016, 7 p. doi.org/10.26181/5d199684490c8

Effects of river flows and temperature on the growth dynamics of Murray cod and golden perch⁵

Key recommendations/options

Species	I	Recommendations	
	Annual discharge	Temperature	
Golden perch Murray	InconclusiveShowed age-	InconclusiveShowed age-specific	NANo single level of annual
cod	 specific responses. Growth of early juveniles (1+ and 2+) peaks at median annual discharge in all rivers, and declines at flows lower or higher than the median. Growth of late juveniles (3+ to 5+) and adults (>5+) increases linearly with annual discharge. 	 responses. The annual temperature (mean daily maximum air temperature) at which maximum growth occurred increased with age, such that growth was maximal at 23 °C, 24 °C and 26–27 °C in early juveniles, late juveniles and adults, respectively. 	 discharge optimal throughout lifetime, so interannual variability in flows must be maintained over the long term. Delivering flows that increase surface of slackwater habitat will enhance growth during early juvenile stage. Delivering flow pulses that increase annual discharge may increase growth of late juvenile and adults. Current climate forecasts for the MDB, coupled with this work, indicate that the forecast rate of warming poses a significant threat to early juveniles, hence recruitment.

Background

To meet Basin Plan objectives and facilitate the Basin Wide Watering Strategy (BWS), scientists must tackle numerous challenges; three of which are particularly pressing. First, we must rapidly develop an understanding of how flow alteration interacts with climate change to affect biota within the Murray-Darling Basin (MDB). Climate change imposes a form of 'non-stationarity' on flows management, in that the flow-ecology rules and decision problems formulated today will be altered by climatic change. This non-stationarity problem is explicitly recognised in the BWS.

⁵ For more detailed information on this project refer to <u>CFE Publication 222/2019</u>.

Second, we must understand how biota respond to the flow-climate interaction at spatial and temporal scales most relevant to management. We need to be able to prioritise and plan environmental flows across multiple catchments within the MDB, and so require general models of ecological response.

Third, our understanding of how climate-flow interactions affect population processes at scales relevant to management must be captured in predictive ecological models. These models are required to: (a) forecast the ecological response to different decision options, leading to better-informed and more defensible environmental flow decisions; (b) facilitate defensible inferences concerning environmental flow impacts under a non-classical experimental design (without controls or replication); (c) improve our capacity to forecast the efficacy of flow management plans under a non-stationary environment; and (d) 'scale up' ecological responses to broader ecological extents or to river segments receiving environmental flows where data are absent or scarce.

Objectives and approach

We used long-term datasets spanning several rivers across the MDB to improve our predictive understanding of how the growth dynamics of two long-lived fishes—Murray cod (*Maccullochella peelii*) and golden perch (*Macquaria ambigua*)—respond to flow variability under a non-stationary climate. Individual growth rate may influence survival and recruitment and, therefore, may be a useful proxy for certain Basin Plan objectives. We had three objectives:

Objective 1 was to estimate growth-rate time series for Murray cod and golden perch in six catchments of the MDB using the information stored in otoliths (fish ear bone). Otoliths were analysed from the Gwydir, Lachlan, Murrumbidgee, Edward-Wakool, Goulburn and Lower Murray systems. Estimating the growth-rate history of an individual fish involves (Figure 8): Step 1, capturing an individual, removing its otoliths, obtaining a slice through the middle of the otolith so that the width of annual growth rings or 'increments' are visible; Step 2, estimating the age of the individual at the time of capture; Step 3, starting from the outer edge of the otolith, measuring increment widths going back through time, such that we may then assign an increment width in an individual to a 'growth year' ($1^{st} Oct - 30^{th} Sept$) and a specific age (Figure 8b). We assume that an individual has not made any large-scale (ca. > 200 km) movements during its lifetime when attempting to relate an individual's growth history to changes in flow and temperature at its place of capture.

Objective 2 was to model growth rates as a function of annual discharge and temperature. 'Annual discharge' was the mean discharge (ML/d) calculated across days of a 'growth year' $(1^{st} \text{ Oct} - 30^{th} \text{ Sept})$. 'Annual temperature' was the mean maximum air temperature calculated across days of the growth year. The modelling approach we used was particularly well suited to situations where inferences or predictions are sought at the population level or 'Basin scale' but where data are sampled from subsets of that population.

Objective 3 was to analyse uncertainty around the growth response to flow and temperature variability. Uncertainty pervades all forms of natural resource management. Improving our understanding of uncertainty around the effects of flows will, in turn, increase our understanding of the range of outcomes that may result from a decision, facilitating more defensible and effective decisions.



Figure 8. Photographs showing how we may obtain the growth history of an individual fish from its otoliths. (a)–(b) Otolith section from a 3+ year-old Murray cod showing key features of otolith increment measurement. Each annual growth increment comprises a pair of translucent and opaque bands that are equal to one year of growth. Broader translucent bands correspond to higher growth periods during warmer months, followed by narrower opaque bands that correspond to lower growth periods during cooler months. Also shown are examples of otolith sections from older individuals: (c) Murray cod age 12+ and (d) golden perch age 19+.

Summary

Effects of annual discharge and temperature on fish growth

Otoliths from a total of 961 Murray cod and 436 golden perch were photographed and analysed. From these otoliths we measured the widths of 4124 and 1957 annual increments from Murray cod and golden perch, respectively. Each of these increments is a measure of the mean growth rate of an individual fish over a year. These sample sizes demonstrate the utility of the biochronology approach to assemble a time series rapidly.

We found no evidence for relationships between golden perch growth and either annual discharge or annual temperature. The most likely explanation for this result is that golden perch undergo significant large-scale movements during their lifetime, which violates the critical assumption stated under Objectives and Approach. There is growing evidence in the MDB for golden perch undergoing relatively large-scale movements during their lifetime.

Annual growth of Murray cod showed significant, age-specific responses to annual discharge and temperature. A visual summary of the key Murray cod results is presented in Figure 9.



Figure 9. Response of Murray cod growth to river flows and temperature - visual summary. Examples from the Murrumbidgee River are presented, but these examples are representative of the MDB as a whole. (a)–(c): Observed annual temperature and discharge time series, as well as the growth time series predicted by the final model (\pm 95% confidence interval) for different life-stages of Murray cod. Vertical dashed lines in (a)–(c) denote La Nina floods and predictions of growth without confidence intervals denote predictions beyond domain of covariates for this river. (d)–(e): predicted growth response to annual discharge (flow percentiles; e.g., Q50 is the median) and annual temperature (°C), respectively, of different life stages. Boxes present the uncertainty in the effect of a covariate (one of annual discharge or temperature) generated by the interaction that covariate has with temporal variation in the other covariate (see CFE Publication 222/2019).

Growth of early juveniles (1+ and 2+) exhibited a unimodal relationship with annual discharge, peaking at the long-term median annual discharge. This is clearly seen in Figure 9d, which presents predicted growth of Murray cod at seven quantiles of annual discharge (Q50 is the median annual discharge; Q25 is the 25th percentile, etc.). In lowland rivers, slackwaters may provide productive foraging habitat for fishes during early life history, and the abundance of slackwaters peaks at

intermediate discharges. One mechanism driving this result may be that energy flow to early juvenile cod is highest at median flows when slackwater abundance peaks.

Contrasting with early juveniles, growth of late juvenile (3+ to 5+) and adult (> 5+) Murray cod increased with annual discharge, with the rate of increase being particularly high in adults (Figure 9d). Higher annual discharge is associated with increased volume of foraging habitat and greater inundation of benches, floodrunners and other floodplain habitat, resulting in mobilisation of energy. Looking at the time series Figure 9c, we can see the strong positive effect of large floods on growth, but also how growth is depressed during the Millennium Drought (ca. 2000–2010).

Unimodal relationships between growth and annual temperature were evident across all Murray cod life stages. The annual temperature at which maximum growth occurred increased with age, such that growth was maximal at 23 °C, 24 °C and 26–27 °C in early juveniles, late juveniles and adults, respectively (Figure 2e). The 'warming' of the thermal niche with age is commonly seen in both marine and freshwater fishes.

Importantly, an analysis of uncertainty clearly showed that the effects of each covariate—annual discharge and temperature—were robust to: (a) environmental uncertainty generated by the interaction one covariate had with stochastic temporal variation in the other, as well as spatial variation among rivers, and (b) statistical uncertainty generated by error in model parameter estimates.

Implications for management of riverine flows in the Basin, in a changing climate

Our study highlights the importance of flows of median annual discharge to growth of early juvenile Murray cod, likely because such flows increase the availability of slackwater habitat for profitable foraging. The distribution and abundance of slackwaters is a consequence of river-floodplain geomorphological dynamics. It follows that, on a decadal scale, the effects of managed flow regimes on the maintenance of vital geomorphological processes should be factored into long-term plans.

This research clearly highlighted the importance of high flows—floods in particular—to growth of late juvenile and adult Murray cod. Increasing the frequency of floods would reduce the probability of hypoxic episodes, while also promoting higher levels of fish productivity.

Given the stage-specific response of Murray cod growth to annual discharge, it is clear that no single level of annual discharge is optimal for Murray cod production. Interannual variability in flows must be maintained to promote production of Murray cod populations over the long-term.

Following the Intergovernmental Panel on Climate Change (IPCC), air temperature records up to 2006 are often used as a 'baseline' for evaluating climate change. Analysis of annual temperatures from our study systems during the baseline period showed that the mean annual temperature was 23.6 °C. This temperature coincided with peak growth of early juvenile cod, while the growth rate of early juveniles declined significantly with warming above the baseline annual temperature. Mean air temperatures are forecast to increase by as much as 5.3 °C by 2090. Given the forecast rate of warming, we may expect depressed growth of juvenile Murray cod under climate change forecasts for the MDB. This may lead to reduced recruitment.

By contrast the data indicate that the warming forecast for the MDB may promote growth of adult Murray cod. Consequently, there is much uncertainty around the overall effect of warming scenarios on Murray cod populations in the MDB. The BWS clearly states the need to quantify the threats posed by climate change. Our work shows that both droughts—through reduction of annual discharge—and warming pose a threat to Murray cod production.

Strategic alignment

This project used established techniques and existing otolith collections, and did not duplicate or overlap with other current projects, especially in the context of relating fish growth to flow and other drivers. All otoliths used by this project have already been collected as part of other projects, most notably LTIM. LTIM otoliths were collected to develop models that enable us to predict age from length, not for the purposes described above in the objectives. Hence, we are maximising the utility of existing collections.

Journal outputs

- Stoffels RJ, Bond NR, and Nicol S (2018). Science to support the management of riverine flows. *Freshwater Biology* **63**(8), 996–1010. <u>doi.org/10.1111/fwb.13061</u>
- Stoffels RJ, Weatherman K, Thiem J, Butler G, Morrongiello J, Bond N, Koster W, Keller Kopf R, McCasker N, Ye Q, Zampatti Z and Broadhurst B (in draft) Impacts of climate flow regime on the growth dynamics of a long-lived riverine fish.

Basin Official Committee questions

Background

The Murray-Darling Basin Officials Committee (BOC) was established by the Murray–Darling Basin Agreement, Schedule 1 to the Water Act 2007. The BOC facilitates cooperation and coordination between the Commonwealth, the Murray–Darling Basin Authority and the Basin states in funding works and managing the Basin water and other natural resources and is responsible for providing advice to the Ministerial Council, and for implementing policy and decisions of the council on matters such as state water shares and the funding and delivery of natural resource management programs.

The objectives of this theme within the MMCP is to help the BOC address specific questions as they arise. These question will have relevance to the on-going management of Basin Assets.

Summary

MMCP is responding to specific questions asked by the BOC relevant to the ongoing management of the MDB system. The responses to these questions are in the form of reviews or synthesis papers. Table 4 provides a list of questions addressed with associated publication numbers for reference to full report.

Table 4. Summary of BOC questions, copies of reports are located on the La Tro	<mark>obe University</mark>	<u>'s data</u>
<u>depository</u> .		

Question	Digital Object Identifiers (DOI)
The potential impacts of climate change on water	• doi.org/10.26181/5d199342d58d5
quality in the southern Murray-Darling Basin	• doi.org/10.26181/5d1eaff43be40
Science to support the management of riverine flows	• doi.org/10.26181/5d199de729a2c
	• <u>doi.org/10.26181/5d1eaf91b823e</u>
Factors contributing to the 2016 hypoxic blackwater	• doi.org/10.26181/5d199d3ce698a
event	• <u>doi.org/10.26181/5d1ad2ac7bddf</u>
How will declining water availability as a	• doi.org/10.26181/5d199c94772e6
consequence of climate change affect habitat and	• <u>doi.org/10.26181/5d1acfd2b3e1e</u>
species distributions in the Murray-Darling Basin?	
Connectivity and floodplain infrastructure	 <u>doi.org/10.26181/5d199f2cda3cf</u>
	• doi.org/10.26181/5d1ad0d670541
Improving our understanding of the ecosystem	• doi.org/10.26181/5d1993d78cf52
effects of varying water levels in weir pools of	• <u>doi.org/10.26181/5d1ad045a241d</u>
lowland floodplain rivers: What role can weir pool	
manipulation play in restoring the health of the	
Murray River Channel?	
eDNA: review of applicability for monitoring and	 <u>doi.org/10.26181/5d1999a3bc14f</u>
detecting biotic populations of the MDB	 <u>doi.org/10.26181/5d1ad38d652fe</u>
Can we better mitigate against weed (aquatic and	• doi.org/10.26181/5d1995c8aa48e
terrestrial) invasions through tailoring flow events in	• doi.org/10.26181/5d19a2e100a80
the Murray-Darling Basin?	

Education

Key outcomes

Number of students supported

The MMCP program has supported:

- Four Summer cadetships
 - o \$4,500 each living allowance
 - o Advertised within LTU only
 - o Three at LTU Albury-Wodonga, one at LTU Mildura
 - Five Honours scholarships
 - o \$5,000 each living allowance
 - o Advertised nationally
 - All at LTU Albury-Wodonga
- Six PhD 'top-up' scholarships
 - o \$10,000 + \$5,000 p.a. scholarships and operating
 - o Advertised both nationally and internationally
 - o Five at LTU Albury-Wodonga, one at LTU Bundoora

International and national partnerships

Of the six PhD top-up scholarships awarded, five were awarded to international students. The benefits of engaging with international students goes beyond the economic benefits to the Australian economy. Professional relationships are formed which provide potential for future international research collaboration and global networking, contributing to expanding the capacity of research in freshwater ecology.

Background

Student support is an important component of MMCP, building research capacity within freshwater ecology. Postgraduate students are able to carry out high-quality research at a reasonable cost and make an important contribution to the development of aquatic research capability. These positions were at either the Albury-Wodonga or Mildura campuses of La Trobe University. Student support is provided through the provision of competitive (merit-based) cadetships, honours scholarships and PhD 'top-up' scholarships.

Cadetships

Cadetships are offered to second-year undergraduate students enrolled within School of Life Sciences programs/units with La Trobe University. During a 10-week summer placement, cadets undertake a small research project on a predetermined topic (Table 5; Appendix 1).

Student	Project Title	Supervisors
Sheree	Gene flow in four macroinvertebrate species in the	Michael Shackleton
Kidziak	Murray River: investigating correlations between	m.shackleton@latrobe.edu.au
	range size, dispersal method and dispersal ability.	
Juliet	The effect of freshwater seed morphology on	Cherie Campbell
Talarico	dispersal and establishment in the Murray–Darling	c.campbell@latrobe.edu.au
	Basin.	
Adriana	Predicting the ability of aquatic and riparian plants	Daryl Nielsen
Galanakis	to disperse.	Daryl.nielsen@csiro.au
		Rebecca Durant
		r.durant@latrobe.edu.au
Matthew	Exploration of symbiotic interactions between	Paul McInerney
Brewer	River Red Gums and mycorrhizal fungi on	p.mcinerney@latrobe.edu.au
	floodplains.	Gavin Rees
		Gavin.rees@csiro.au

Honours scholarships

Honours scholarships are awarded through a merit selection process and advertised nationally to attract suitably qualified candidates (Table 6; Appendix 1). Copies of these completed thesis are stored on La Trobe University's data depository.

Table 6. Honours scholarships undertaken as par	rt of MMCP student funding.
---	-----------------------------

Student	Project Title	Supervisor and Thesis location
Sheree	The ability of PCR-based methods and next	Michael Shackleton
Kidziak	generation sequencing to detect secondary	m.shackleton@latrobe.edu.au
	predation in the gut contents of aquatic	doi.org/10.26181/5d1eb2326c086
	macroinvertebrates.	
Nissa	The effect of waterhole permanency and	Amina Price
Davis	hydrological connectivity on fish community	a.price@latrobe.edu.au
	composition in a dryland river.	doi.org/10.26181/5d1eb2f1b13ef
James	Stress tolerance losses from stabilising	Michael Shackleton
O'Dwyer	selection in a salt lake organism.	m.shackleton@latrobe.edu.au
		Nick Murphy
		n.murphy@latrobe.edu.au
		doi.org/10.26181/5d1ebff03ad1f
Kate Hill	Understanding the functional connectivity of	Daryl Nielsen
	non-floodplain wetland plant populations in an	Daryl.nielsen@csiro.au
	agricultural landscape.	Michael Shackleton
		m.shackleton@latrobe.edu.au
		doi.org/10.26181/5d1eb2326c086
Teresa	Adverse effects of microplastics observed on	Aleicia Holland
Calipari	the growth rate and health of the freshwater	a.holland2@latrobe.edu.au
	alga, Chlorella sp. 12.	doi.org/10.26181/5d1ec182ca689

PhD scholarships

PhD 'top-up' scholarships were awarded through a merit selection process and advertised nationally and internationally to attract suitably qualified candidates. Prospective students were required to be eligible for, and obtain an Australian Postgraduate Award or an International Postgraduate Research Scholarship and enrol in La Trobe University. These postgraduate studies are still ongoing and as such no postgraduate theses have been completed (Table 7; Appendix 1), on completion students will also develop a summary factsheet. Both the PhD thesis, factsheet and any papers that are derived from each of these thesis, will be distributed to the MDBA and the JGR as well made available on the La Trobe University's data depository.

Student	Project title	Completion date
Lorena	Water infrastructure and challenges for fish conservation: A	July 2019
Nogueira	trait-based analysis to foresee fish recruitment in regulated	
	rivers.	
Manisha	Impact of environmental stress on the protein and amino acid	February
Shakya	composition of freshwater organisms	2020
Francesco	Legacy effects of historical gold mining on floodplains of	February
Colombi	Victorian rivers	2020
James	Flows for fish: Using water flow to promote connectivity,	February
O'Dwyer	recruitment and genetic diversity for Australian fish species.	2021
Lucas Morais	The influence of water chemistry on zinc bioavailability and	November
	toxicity in Australian freshwaters.	2021
Summan	Dissolved organic nitrogen in natural waters as a source of	June 2022
Acharya [*]	bioavailable nitrogen.	

Table 7. PhD	Scholarships	undertaken a	s part of MMCF	student funding.
--------------	--------------	--------------	----------------	------------------

*Commences in July 2019, title of project subject to change.

Communication

Background

The ultimate goal of the communication section of MMCP was to identify key stakeholders and ways to communicate effectively with them to raise the overall awareness of the project and its outcomes. All relevant outputs will be made available on <u>La Trobe University's data depository</u>.

Summary

A number of activities (refer to Appendix 2) have been undertaken as part of the overall communication strategy, these outputs include:

- Annual reports.
- Published peer-review journal article.
- BOC question synthesis.
- Student theses (summer cadetship and honours).
- Conferences, seminars and presentations.
- Annual forums.
- Centre/University E-newsletters.
- Social media and media releases.
- Published factsheets.

Appendix 1: Education Overview

Introduction

Student support is an important component of MMCP. Postgraduate students carry out high-quality research at a reasonable cost and make an important contribution to the development of aquatic research capability. These positions were embedded in the Centre for Freshwater Ecosystems at either the Albury-Wodonga or Mildura campuses of La Trobe University. Student support is provided through the provision of cadetships, honours scholarships and PhD 'top-up' scholarships.

Cadetships

Cadetships were offered to second-year undergraduate students enrolled within School of Life Sciences programs/units within La Trobe University. During a 10-week summer placement, cadets undertook a small research project on a predetermined topic. Four cadetships were undertaken between the summer of 2015 and 2018. This section provides the executive summary or introduction to each of the student's reports.

Sheree Kidziak

Project title: Gene flow in four macroinvertebrate species in the Murray River: investigating correlations between range size, dispersal method and dispersal ability.

Supervisor: Michael Shackleton, La Trobe University, Wodonga Vic.

Abstract: The ability to disperse between populations is important for maintaining the health and dynamics of species' populations. Dispersal leads to greater gene flow between populations, which in turn results in higher resistance to change. Dispersal can be impacted by different factors, such as fragmentation of habitat. Changes in a species dispersal patterns can also regularly be influenced by changes within landscapes. A good understanding of how individuals disperse is important when studying population interactions. For aquatic species, there is a general assumption made that dispersal mode is closely linked to dispersal ability. The ability to fly is seen as an advantage to overcoming barriers that could prevent connectivity between populations. By contrast, species that are confined to instream habitats are seen as limited in their ability to distribute as effectively. Another common assumption is that a species ability to disperse will be directly related to its range size. It is theorized that if a species has the ability to inhabit new territory, it will. This leads to the inference that species with better dispersal abilities will have larger range sizes. However, this assumes that there are no external factors influencing distributions, such as environmental constraints. However, species with good dispersal ability but restricted in their ability to expand their range size, should exhibit low levels of inbreeding, and a high transfer of genes across populations, thus leading to panmixis. Estimations of dispersal in freshwater invertebrates are often problematic. Direct methods of tracking dispersal, such as observation of behaviours, or light trapping, have had little success in regards to measuring dispersal. However, measuring the exchange of alleles between populations has proven to be an effective method for inferring dispersal interaction between populations. Gene flow gives an indication of how genes move through populations, and thus how commonly populations interact with each other. It is expected that fragmented populations are more genetically differentiated than populations that are well connected. This study explored the relationship between dispersal, range size and dispersal mode in four aquatic invertebrates. In particular, this study investigated two questions: (i) Do species with larger range sizes disperse better than species with a smaller range size?; and (ii) Do species that fly disperse more readily than

species that are confined to instream environments? To answer these questions, four species were chosen for investigation. Two species were confined to instream environments (*Paratya australiensis* Kemp, 1917, and *Macrobrachium australiense* Holthius, 1950) and two were able to fly (*Ecnomus pansus* Neboiss, 1982, and *Micronecta annae* Kirkaldy, 1905). Species selection also encompassed two species with large ranges sizes and two with more restricted distributions. Strength of dispersal was inferred by comparing genetic divergence between populations using Fst values. It was hypothesized that (1) The two river confined species, *P. australiensis* and *M. australiense* would be better dispersers compared to *M. annae* and *E. pansus* which have the ability to fly, and (2) That species with larger distribution sizes, *M. australiense* and *E. pansus*, would show smaller Fst values compared to the species with smaller distributions, *P. australiensis* and *M. annae*.

Julit Talarico

Project title: The effect of freshwater seed morphology on dispersal and establishment in the Murray–Darling Basin.

Supervisor: Cherie Campbell, La Trobe University, Mildura Vic.

Executive summary: Seeds are a crucial part of plant taxonomy, yet there are limited identification resources for them, notably in the Mallee region. This research focused on macrophyte species occurring in the Murray–Darling Basin of the Victoria/New South Wales region. The objective was to develop a catalogue of seed images from fresh and dry herbarium specimens. Images provided information of the seeds physical attributes and this was used to determine their potential to disperse across landscapes and establish a plant system. Fresh seeds were compared with dry seeds of the same species and it was hypothesised that dry samples would have a lower mass, surface area, volume and density. Lignum (*Duma florulenta* Meisn.) and Slender Dock (*Rumex brownii* Campd.) were of particular interest. A one-tailed t-test was used to infer if hypotheses were supported. Findings indicated that all measured traits decreased in the dry samples except density, which increased. All data were regarded statistically significant other than mass (of both species) and Slender Dock density.

Adriana Galanakis

Project title: Predicting the ability of aquatic and riparian plants to disperse.

Supervisor: Daryl Nielsen, CSIRO Land and Water, Albury NSW; Rebecca Durant La Trobe University, Wodonga Vic.

Executive summary: This study is a part of a larger vegetation dispersal project being conducted by the Murray–Darling Freshwater Research Centre (MDFRC). The objective of the project was to understand how the influence of flow characteristics associated with water as a dispersal vector influence the dispersal of propagules by water. In this study, we examined the physical and morphological characteristics of aquatic and riparian seeds to establish a relationship to their ability to float. The seeds of seven species were collected and their length, width, height, weight, surface area, volume, density and shape were measured. A buoyancy trial was conducted over a four-week period to determine their ability to float. The conclusion of this study was that the length, surface area and shape of the seed affects seed buoyancy. The aim of this study was to increase knowledge on the morphological characteristics that increase the ability of a seed to float. Therefore enabling predictions on where the seeds are more likely to occur in the drift. The results will help us understand how infrastructure can potentially influence the dispersal of seeds.

Matthew Brewer

Project title: Exploration of symbiotic interactions between River Red Gums and mycorrhizal fungi on floodplains.

Supervisor: Paul McInerney, La Trobe University, Wodonga Vic.; Gavin Rees, CSIRO Land and Water, Albury, NSW>

Introduction: The Murray–Darling Basin (MDB) is an economically and environmentally important area in Australia, accounting for 20% of the nation's total agricultural land and producing one-third of the nation's food supply. A vast majority of the MDB is flat and low-lying with water sourced from high rainfall regions in the east, primarily the mountains in the Great Dividing Range. The MDB contains a network of rivers and floodplains encompassing approximately 30 000 wetlands, eleven of which are protected by the Wetlands of International Importance Act because of their contribution to the ecosystem. A floodplain may be defined as an area of land adjacent to a river or stream that is low-lying and subject to flooding during high flows in rivers. Subsequently, floodplain soils typically contain a high proportion of organic materials deposited from flood waters. Inundation increases connectivity between aquatic habitats and facilitates the exchange of nutrients (N and P), carbon and biota. This exchange alters the chemical composition of the environment affecting pH and organic content, which impacts the composition of microbial communities in the soil. Alterations in chemical content in an ecosystem affects the floral diversity, carbon dioxide production and nutrient composition, which inadvertently changes soil fungal diversity. Fungi are important both to agricultural and natural ecosystems, since they directly impact the quality of the soil and play a vital role in nutrient recycling. Agriculture is the primary industry within the MDB and consequently, fungal communities associated with commercially important agriculture are well studied. However, research exploring soil microbial communities in floodplain ecosystems is less advanced, particularly within pastures previously dominated by native riparian plants (e.g. Eucalyptus camaldulensis). Differences in microbial communities from areas of differing agricultural land use and intensity have been documented before and historical land-use may be an important driver for community structure and may assist with possible future revegetation projects. Terrestrial forest environments contain the highest fungal diversity, partly due to mutualistic associations formed between trees and mycorrhiza. High diversity may also be attributed to reduced anthropogenic activity, since fungi are most diverse in minimally disturbed ecosystems. Basidiomycota are a division of fungi known to form mycorrhizal associations with plants. A mycorrhizal association is a mutualistic relationship between fungi and plants where the former promotes the uptake of nutrients and the latter receives organic carbon in return. Fungi facilitate nutrient uptake by increasing surface area of the roots and releasing chemicals into the rhizosphere, dissolving nutrients bound to the soil. These mechanisms allow plants to tolerate drought and high temperature environments, and stimulate growth in nutrient poor soils. Agricultural fields exposed to high levels of land use show the lowest level of fungal distribution due to the effects of irrigation and livestock. Fields subject to periodic livestock grazing are dominated by Ascomycota, a division of fungi that produce both mycorrhiza and saprophytes. Saprophytic fungi are the largest group of fungi. They decompose dead organic matter and recycle the nutrients by degrading complex molecules, making them available for absorption for other organisms. The aim of this study is to investigate fungal communities inhabiting grazed and undisturbed floodplain E. camaldulensis forest soils in comparison to communities from adjacent pasture soils that have been cleared for grazing. We expect that E. camaldulensis forest soils will contain more mycorrhiza fungi than the pasture soils as the trees have been cleared. We also predict that the forest void of cattle will display higher fungal diversity due to decreased disturbance.

Honours scholarships

Honours scholarships were awarded through a merit selection process and advertised nationally. MMCP funded five honours scholarships.

Sheree Kidzaik

Project title: The ability of PCR-based methods and next generation sequencing to detect secondary predation in the gut contents of aquatic macroinvertebrates.

Supervisor: Michael Shackleton, La Trobe University, Wodonga Vic.

Abstract: This study is the first to use Next Generation Sequencing (NGS) to investigate the distinction between primarily- and secondarily-consumed prey items in gut content analyses of aquatic macroinvertebrates. Understanding the level at which secondary predation is accounted for in dietary studies is vital for preventing inferences of false trophic links in food web studies. This study investigated the detectability of secondary predation by conducting feeding trials using a Chironomidae-Corixidae-Odonata system. The gut contents from these trials were tested using PCR-based amplification and NGS.

Results from NGS were sporadic and significantly less DNA sequences were returned than expected, which indicated that there were some issues that prevented PCR amplification from occurring. From ratio interaction tests, it appeared that larger concentrations of Corixidae DNA in samples seemed to cause PCR amplification to fail. Dilution tests indicated that it was not necessarily the biomass of the taxa that determines the success of amplification, rather the concentrations of DNA for each individual present in the sample.

While this method failed to successfully identify a distinction between primarily- and secondarilyconsumed prey items, it highlights critically important issues with using NGS in metagenomics studies. The issues explored in this study have the potential to cause concern across a wide range of uses in genetic investigations. This study lays the foundation work for further investigations to continue to investigate a way of determining secondary predation. Additionally, this study highlights the need of additional research into food webs and dietary assessments on aquatic macroinvertebrates.

Nissa Davis

Project title: The effect of waterhole permanency and hydrological connectivity on fish community composition in a dryland river.

Supervisor: Amina Price. La Trobe University, Wodonga Vic.

Abstract: Fish community composition is influenced by a range of variables, including water quality, habitat availability and complexity, and primary production. In dryland rivers, which periodically disconnect into isolated waterholes, variables such as waterhole permanency and hydrological connectivity, as well as spatial context, are likely to be more important than in perennial river systems. Previous studies in dryland river systems have generally focussed on permanent waterholes and consequently, the importance of variables such as waterhole permanency has not been assessed.

The aims of this study are to examine: (1) fish assemblages across a range of waterholes with varying levels of permanency; (2) fish assemblages during different levels of hydrological connectivity (i.e.

waterholes connected and waterholes disconnected; and (3) the relative influence of local and regional factors on fish community composition.

The overall composition of fish communities differed significantly between dry and wet periods. During the dry periods, there was a higher abundance of alien species than native species and larger body-sized fish, whilst during the wet periods there was a higher abundance of juvenile and smallbodied native fish. Fish community composition significantly differed between ephemeral waterholes and permanent waterholes, with *Maccullochella peeli*, *Ambassis agassizii*, *Hypseleotris* spp. and *Neosilurus hyrtlii* only collected in semi-permanent to ephemeral waterholes.

Environmental analysis of the local variables showed that 58.8% of variation between wet and dry and community composition was influenced by waterhole length and width, temperature, turbidity, dissolved oxygen and dissolved oxygen %. Regional variables (spatial context, distance between waterholes and between permanent waterholes) showed no significance in the differences between fish community composition.

The findings of this study highlight the importance of the permanency gradient, specifically the potential role of ephemeral waterholes and hydrological connectivity in structuring fish community assemblages in dryland rivers.

James O'Dwyer

Project title: Stress tolerance losses from stabilising selection in a salt lake organism.

Supervisor: Michael Shackleton, La Trobe University, Wodonga Vic.; Nick Murphy, La Trobe University Bundoora, Vic.

Abstract: The capacity of a population to tolerate and resist physical stressors is crucial for survival inside any environment, with all species being at least partially restricted to environments based on what abiotic niches they can and cannot occupy. Stabilising selection is thought to play a crucial role in reducing phenotypic variance for stress resistance traits, but very few studies have demonstrated this occurring in natural systems. The main objective of this study was to use multiple populations of a salt lake snail (Coxiella striata) from both stable and fluctuating environments to determine if stabilising selection leads to an overall reduction of stress resistances within a population. Samples were collected from two stable and two unstable salt lakes in Victoria, Australia, where stability was determined as long-term, consistent salinity concentrations in each lake. Each population was then exposed to a variety of salinity, desiccation and temperature stress tests, for both prolonged exposure to, and gradual changing degree of stressors. Samples were also genetically sequenced for the mitochondrial regions CO1, and 16s, and the nuclear gene ITS, and each population was shown to be genetically distinct. Snail populations from the stable lakes showed a significantly lower survival rate to salinity and temperature tests, while also showing a larger size for two key morphological traits within the species, shell length and operculum width. The results of this study support the hypothesis, that stabilising selection has led to a clear reduction in stress tolerances for populations of C. striata found in stable environments. This is an important finding when considering the ramifications of reduced stress resistance traits in a rapidly changing climate.

Kate Hill

Project title. Understanding the functional connectivity of non-floodplain wetland plant populations in an agricultural landscape.

Supervisor: Daryl Nielsen CSIRO Land and Water, Albury NSW; Michael Shackleton, La Trobe University, Wodonga Vic.

Abstract: In Australia, little attention has been given to ascertain the functional connectivity of wetland plant communities, that is, the actual dispersal of individuals or genes amongst wetland plant populations. To determine the levels of functional connectivity among a network of non-floodplain wetlands in an agricultural landscape, genomic DNA was extracted and sequenced from leaf tissue samples of two common wetland macrophyte species (*Carex tereticaulis* and *Myriophyllum crispatum*) within the lower Ovens River valley of northeast Victoria (south-east Australia). Using genome-wide single nucleotide polymorphisms data sets for each species, we investigated fine-scale population genetic structure across the study area. It was hypothesised that wetland plant populations would exhibit high levels of functional connectivity (and/or gene flow), despite being surrounded by an agricultural matrix due to the potential for highly mobile dispersal vectors to widely disperse propagules. Quantifying the successful dispersal and establishment of individuals between extant wetland macrophyte populations is important for understanding the mechanisms that promote a species long-term persistence and evolutionary potential to cope with anthropogenic modification, habitat fragmentation and climate change.

Both species exhibited low levels of functional connectivity among the wetland populations, with some evidence of spatial genetic structure by individual wetland population. It is likely that agricultural landuse change over the past 100 years is driving low, but significant levels of genetic differentiation in allele frequencies among all wetland populations (pairwise Fst) for both species due to reduced genetic exchange. Low levels of genetic differentiation suggests there may be occasional successful long-distance dispersal or migration, but currently there is not enough to create any significant genetic similarities among the populations. Inter-population genetic drift via random loss of specific alleles is most likely the main force shaping the spatial genetic structure of both species. Current dispersal pathways for gene flow appear restricted, including among those wetlands that are immediately connected hydrologically and/or distributed over a relatively small distance (<8 km apart). Consequently, the impact of fragmentation on genetic diversity will probably become more evident in the future given the long-lived life history of both species. These results highlight the importance of each sampled wetland population to maintaining the overall genetic diversity of both species, and the metapopulation system as a whole. Increasing the protection of each wetland and their connecting drainage lines is recommended to improve the long-term genetic stability of these wetland macrophyte populations. These findings contribute to our understanding of the biological connectivity of wetland plants in Australian landscapes, which is important to generate accurate predictive models for conservation and management in the light of rapid environmental change.

Teresa Calipari

Project title: Adverse effects of microplastics observed on the growth rate and health of the freshwater alga, *Chlorella* sp. 12.

Supervisor: Aleicia Holland, La Trobe University, Wodonga Vic.

Abstract: Microplastic pollution in freshwater systems has gained significant attention in recent years. There have been numerous studies looking at the concentrations, and biological and environmental impacts of microplastics (MP) in freshwater ecosystems. This study aims to add to the growing body of literature surrounding the biological and toxicological effects of MPs by exploring the effects they have on the growth and health of the freshwater alga, *Chlorella* sp. 12. Algae were

exposed to increasing concentrations of polyethylene MPs (0-1000 mg.L⁻¹) in 72 hour bioassays. EC10 and EC50 values occurred at 220 (181–259: 95% confidence limits) and 283 (247–319) mg.L⁻¹, respectively. There was a significant reduction in the growth rate of algae exposed to high concentrations of MPs compared to controls. No significant differences occurred at concentrations <375 mg.L⁻¹. The addition of a common freshwater pollutant, Copper (Cu; 1 µg.L⁻¹), enhanced the toxic effects on *Chlorella* at high MP concentrations. Two environmentally realistic concentrations of polypropylene MP (760 and 2733 particles.L⁻¹- derived from current literature) were used to observe changes in cell health; that is, chlorophyll fluorescence and amino acid composition. High MP concentrations (2733 particles.L⁻¹) showed a 20% reduction in growth rates of algae when compared to controls. No significant differences in the relative abundances of some amino acid when exposed to high MP concentrations. There was no evidence of hetero-aggregate formation between algal cells and MPs. This study is the first to provide EC10 and EC50 values for this alga when exposed to MPs, and shows that algal cell health is affected at environmentally realistic concentrations.

PhD scholarships

Top-up scholarships were awarded through a merit selection process and advertised nationally and internationally. Prospective students were required to be eligible for, and obtain an Australian Postgraduate Award or an International Postgraduate Research Scholarship and enrol in La Trobe University. MMCP have funded five postgraduate scholarships. These postgraduate studies are still ongoing and as such no thesis is completed. Below are summaries of each student's project and progress.

Lorena Nogueira

Due for completion: July 2019

Project title: Water infrastructure and challenges for fish conservation: A trait-based analysis to foresee fish recruitment in regulated rivers.

Supervisors: Amina Price¹, Susan Lawler¹, Paul Humphries² and Lee Baumgartner²

Affiliations: ¹La Trobe University, Wodonga, Vic; ²Charles Sturt University, Albury, NSW.

Background

Dispersal of early life-stages of fish, moving through critical habitats, is an essential component of the life cycle of many freshwater fish species. Despite the significance of this element to species ecology, knowledge of the processes and patterns of freshwater larval fish dispersal is scant. The larval phases are generally characterised by poorly developed swimming and sensorial abilities. Therefore, they are especially susceptible to habitat alteration. The slow-flowing habitat created by reservoirs and weir pools can interact with larval swimming behaviour causing loss of orientation and preventing them from dispersing to appropriate nursery areas and other critical habitats, mainly if their movement is dependent on flow cues. Indeed, for some time, it has been assumed that the larvae of many species disperse passively. However, there is growing evidence showing that dispersal for some species may be active for at least part of the early developmental stages. This becomes paramount information to enlighten management and mitigation measures for flow-modified systems, such as the establishment of environmental flows. In Australia, flows within the Murray–Darling Basin have been profoundly altered by multiple usages, and concerns have been long-standing in regards to the effects of such modifications on native fish populations.

Nevertheless, there is still a significant gap to the understanding of the impacts of such flow alterations on larvae.

The present study aimed to improve the understanding of larval dispersal of three iconic Australian freshwater fish, Golden perch (*Macquaria ambigua* Richardson), Murray cod (*Maccullochella peelii*), and Trout cod (*Maccullochella macquariensis* Cuvier, 1829), by investigating swimming behaviour (swimming activity, orientation towards the current vector and direction of movement) under a range of simulated flow conditions.

Preliminary results

Successful experiments were performed for all life stages of Murray cod and Trout cod. The swimming experiments with the two first life stages of Golden perch, preflexion and post-flexion, could not be achieved because of their small size and lack of body pigmentation, which made their visualisation in the racetrack flume extremely difficult.

Initial results of the swimming behaviour showed that during downstream movements, the majority of individuals were oriented upstream, with active swimming behaviour in the assessed fish species. In this movement pattern, named active-passive, the fish moves at a slower rate than the flow and can change its position between the fast and slow-flowing areas of the racetrack flume. Active upstream behaviour was observed for all three species, with more significant distances performed by the older life stages. Active-downstream movements (downstream orientation) were also identified. The active nature of larval dispersal showed by young individuals of Golden perch, Murray cod, and Trout cod, underpin the need to improve our understanding of the relationship between fish larvae and the riverine environment. This is certainly paramount for effective river management and assessment of impacts caused by water infrastructure development.

Future activities

A different approach, using drift nets, will be tested for the first stages of Golden perch (preflexion and post-flexion larvae), as an attempt to determine the active or passive nature of their movement. Also, a hydraulic model for the racetrack flume is in preparation, and it will be used for the complete data analysis of the swimming experiments.

Manisha Shakya

Due for completion: February 2020

Project title: Impact of environmental stress on the protein and amino acid composition of freshwater organisms.

Supervisors: Ewen Silvester¹, Gavin Rees² and Aleicia Hollands¹

Affiliations: ¹La Trobe University, Wodonga, Vic; ²CSIRO, Land and Water, Albury, NSW.

Executive summary: Amino acids (AA) are one of the main bio-molecule types transferred across aquatic food web. AAs consist of essential and non-essential AAs; essential AAs must be obtained through an organism's diet, whereas, non-essential AAs can be synthesised by an organism. AAs regulate key metabolic pathways that are crucial to maintenance, growth, reproduction and immune responses and serve as a metabolic substrate for the production of energy. AAs are also selectively incorporated into the tissues of organisms as proteins and are therefore important with respect to the nutritional benefit offered to consumer organisms. While the AA composition of a prey organism likely varies between taxa, it may also vary within taxa in response to: differences in the

environments in which they dwell, the biogeographical region they live in and/or specific attributes at the sites where they are found. Such changes to AA composition at the individual level may have cascading effects on the overall aquatic food web. As macroinvertebrates are a major prey item for higher organisms such as fish, a change in the macroinvertebrate composition could lead to a change in the nutritional landscape available for consumers.

Freshwater organisms are often exposed to different environmental and anthropogenic stressors, through the alteration of physical and chemical conditions within water bodies. Exposure to such stressors can cause adverse effects to the organisms living in the water bodies, which may be observed in the different ecological levels of organisation, such as individual, population, community and ecosystem. Environmental stressors and contaminants exhibit their toxic effects by altering cellular and subcellular processes within individual organisms, before any effects to an organism's growth, reproduction and/or survival are observed. For this reason, changes in biomarkers such as AAs, have been suggested as a sensitive tool to assess exposure to environmental and anthropogenic stressors. Such measures are useful to explore the impact of stress at the individual level and understand the interaction between changes in water quality and freshwater organisms. However, information regarding the influence of environmental stressors such as temperature and contaminants on protein and total AA profiles (i.e. protein + peptide + free AAs) of aquatic organisms is currently limited. This project aimed to explore the effects of environmental and anthropogenic stressors on the amino acid composition of freshwater organisms using both field and laboratory experiments.

The first objective of the project was to explore the variation in the amino acid composition of macroinvertebrate taxa collected from seven sites along the Murray River, Australia. The target species of this study were *Macrobrachium australiense*, *Paratya australiensis*, *Physa* spp. and *Triplectides* spp. The work provides the first comprehensive study about the total AA composition of macroinvertebrates (four taxa) in the Murray–Darling Basin in order to understand the variation between taxa. The work has further focused on linking changes in the amino acid composition of taxa with changes in environmental variables between sites and season (summer and winter) to better understand how these factors shape the amino acid composition of selected macroinvertebrates of the Murray River.

The four taxa were shown to have distinct AA profiles, which significantly differed from each other (Figure 10a). Our results also showed that the AA profile of decapods (*M. australiense, P. australiensis*) significantly varied seasonally between summer and winter but this was not the case for the *Physa* spp. or *Triplectides* spp.

For both decapods, the AA profiles showed a seasonal variation in glycine content, with higher levels during winter. Proline and serine content also varied in *P. australiensis* between summer and winter, but this was not observed in *M. australiense*. Water temperature was the main physical parameter that varied significantly between summer and winter, with a difference of around 10 °C across all sites. This suggests that temperature may be one of the environmental variables that influences the AA composition of the decapods. No significant site effect was observed in the amino acid composition of *M. australiense* and *P. australiensis* despite these organisms being collected from seven different sites along the Murray River and having different water qualities (based on the spot data on temperature, DO, pH, turbidity and EC collected during the sampling period). To further investigate the potential effects of temperature on the amino acid composition of aquatic organisms, laboratory trials will be conducted. Algae, shrimp and fish will be exposed to different temperatures and their amino acid composition determined. These three organisms were chosen to represent different trophic levels within the aquatic environment.



Figure 10. Multidimensional scaling (MDS) ordination based on 95% confidence interval bootstrap average showing a significant difference in amino acid profile among different taxa from the a) Murray River, Victoria, and b) Dee River, Queensland.

The second objective of this research was to investigate how metal contamination within river systems alter the amino acid composition of freshwater organisms. Macroinvertebrates were collected from seven sites along a contamination gradient (Dee River, Queensland: three polluted sites, two controlled sites (upstream) and two sites downstream). Temperature, dissolved oxygen, salinity, conductivity and pH were recorded along with concentrations of metals. Polluted sites were heavily contaminated with metals such as cadmium, copper, iron, nickel and zinc, with their values exceeding the Australian and New Zealand Guidelines for Fresh and Marine Water Quality. Analysis of total AA composition in whole body tissues of five different insect taxa: Chironomidae, Ceratopogonidae, Dytscidae, Gomphidae and damselfly showed that AA composition differed between taxa (Figure 10b). Furthermore, the AA profile of Diptera (Chironomidae, Ceratopogonidae) and Coleoptera (Dytscidae) significantly differed between control sites (upstream) and contaminated sites (polluted sites and downstream). However, such variation was not observed in Odonata (Gomphidae or damselfly) (Figure 11).





To explore the relationship between changes in amino acids and metals, further laboratory experiments exposing organisms (algae; Chlorella sp.), shrimp and fish (Southern purple-spotted gudgeon (PSG); Mogurnda adspersa Castelnau 1878) to copper will be conducted. To date, the impact of copper on the AA profile of Chlorella sp. and sac fry of Southern purple-spotted gudgeon has been conducted. Firstly, the effects of copper on the growth rate and AA profile of Chlorella sp. were determined in 72 hour exposure experiments at environmentally realistic copper concentrations over the range $1-10 \ \mu g.L^{-1}$. The copper concentration required to inhibit algal growth rate by 10 % (EC10) and 50 % (EC50) was found to be 1.0 \pm 0.1 μ g.L⁻¹ and 2.0 \pm 0.1 μ g.L⁻¹, respectively. Copper stress caused a significant change in the AA profile of *Chlorella* sp. compared to the controls, with an increase in some AAs, including: Glx (glutamine + glutamic acid) and glycine and decreases in: arginine, threonine, proline, valine, leucine, phenylalanine, isoleucine, leucine, with increasing copper concentrations. Similarly, 96 hour acute toxicity tests were used to determine the EC10 and EC50 (ECx: effective concentration that causes loss of equilibrium in x% of test organism) values for PSG sac-fry. The impact of copper on the protein content and amino acid profile of sac-fry after 96 hour exposure was also investigated. The EC10 and EC50 (based on loss of equilibrium) were found to be $12 \pm 2 \mu g.L^{-1}$ and $22 \pm 1 \mu g.L^{-1}$, respectively. Copper stress caused a significant change in the protein content and amino acid profile of the sac-fry of PSG compared to the controls with decreased: ASx (aspartic acid+ asparagine) and arginine and increased: proline, serine, threonine, valine, tyrosine, isoleucine, leucine and phenylalanine, with increasing copper concentration (stress). The proportions of sulfur-rich AAs: cystine and methionine, remained unaffected within the range of copper concentrations tested (5–60 µg.L⁻¹). In the future, similar experiments will be conducted using shrimps.

Future work will involve the use of proteomics to understand what the changes in AA profiles may mean in respect to the function of an organism. In addition to this, synchrotron-based infrared (FTIR) microscopy and proton-induced X-ray emission (PIXE) will be used to provide supporting evidence for the co-association of proteaceous materials in regions which accumulate copper within the PSG sac-fry. The proposal for this work has been accepted by Australian Synchrotron and will be conducted early August 2019. To the best of our knowledge, this is the first study to explore changes in AA profiles of aquatic organisms in response to the environment within Australian river systems and the first to explore possible effects of the environmental stressors such as temperature and copper on aquatic organisms at various trophic levels.

Francesco Colombi

Project Title: Legacy effects of historical gold mining on floodplains of Victorian rivers

Due for completion: February 2020

Supervisors: Ewen Silvester

Affiliations: La Trobe University, Wodonga, Vic.

Abstract: This PhD project is part of an ARC-funded project (Rivers of Gold) that is a collaboration between archaeologists, geomorphologists and geochemists from La Trobe University (Centre for freshwater Ecosystems), University of Melbourne and Lincoln University (UK). The application of these diverse disciplines is intended to achieve a more complete understanding of the impact of gold mining in Victoria. Gold mining in Victoria during the nineteenth century (1851-1950's) was characterized by the exploitation of gold resources varying in nature from gold-rich sulphidic ore bodies to alluvial deposits (placers). The technologies adopted for the processing of gold-bearing materials evolved with the types of deposit being mined. Because of the lack of any sort of regulation for the containment of mine wastes, the tailings produced by the recovery of gold ores were often released directly into aquatic ecosystems and surroundings. The production and subsequent downstream re-distribution of these tailing wastes is still visible today on Victorian river banks as revealed by the presence of yellowish, sandy and silty layered deposits, lying on top of an earlier floodplain (Figure 12).



Figure 12. Photograph of mine tailings overlying original floodplain surface (marked by red line; Loddon River).

Soils and sediments, especially in mined-affected environments, may act as sink or source for pollutants. This project aims to determine the distribution of contaminant metals and metalloids (e.g. As) through field and laboratory analysis of soil sediments (river bank deposits) and to understand the geochemical changes that have occurred in these tailing deposits. The Loddon River is one of the most mining-affected catchments in Victoria and has been a particular focus of this work.

To assess the degree of contamination affecting the Loddon River catchment, the following aspects have been investigated in this project: 1) characterization of the physical and geochemical changes that occurred in mine tailings after re-deposition on downstream floodplains and comparison of these tailings with the (relic) underlying floodplain sediments; and 2) understanding the chemical form of the re-deposited tailings in order to evaluate their potential bioavailability and environmental fate in these deposits. A number of sites along the Loddon River have been investigated, both upstream and downstream from the main mining areas. River bank deposits were analysed directly using a portable X-ray fluorescence (p-XRF) spectrometer and sampled for further laboratory analysis. In-field X-ray measurements are a powerful tool allowing a direct evaluation of the sediment elemental composition and potentially an immediate distinction between the tailings and the underlying floodplain. In the laboratory, these sediments have been characterised for particle size distribution (texture) using dry sieving and laser sizing (Department of Geography at Melbourne University). Loss on ignition (LOI; organic carbon content) has been measured as a parameter to distinguish original floodplains from as well as to determine the extent of top-soil reformation. Total arsenic concentration in the laboratory has been determined by chemical digestion coupled with graphite furnace atomic absorption spectroscopy (GF-AAS); arsenic is the most abundant contaminant metal (metalloid) found in these tailings and has been used as a proxy to differentiate the tailings from the relic floodplain. Laboratory measured levels of As have been compared with field p-XRF to determine the reliability of in-field measurements.

The work has identified the presence of an arsenic rich 'plume' (around 1 metre below the current surface) containing arsenic levels as high as 300 ppm (mg-As/kg), significantly higher than both the low level (20 ppm) and high level (70 ppm) interim sediment quality guidelines (ISQG) for Australian and New Zealand (Figure 13).

This submerged plume is located above the original floodplain surface and occurs downstream of the main mining areas of the catchment. The upstream sites show a sharp increase in organic carbon content at the transition between tailings and underlying original floodplain, suggesting carbon rich ('swampy') pre-European environments. A differentiation in between tailings and the underlying (relic) floodplain is also indicated by texture, with the anthropogenic sediments having a generally finer size and more variability in grain-size distribution.

As these As-contaminated sediments are upstream of major reservoirs and distributed widely across floodplains, understanding the chemical forms and mechanisms of mobilization of the metalloid is fundamental to improving our understanding of how such disturbances modify river systems. Consequently, the second objective of this work has involved further investigation of the geochemical changes which have occurred to the mobilised parent minerals through river transport and exposure to the atmosphere. For this we have used electron microprobe analysis (EPMA) and X-ray absorption spectroscopy (XAS) for the analysis of As-containing particles; high-As samples were selected for EPMA (>100ppm) and XAS (>50ppm) analysis. Electron microprobe backscattered micrographs show that arsenic is found strongly associated with highly porous and cemented iron oxides phases at levels up to 5%. From our preliminary results, XAS studies reveal that the dominant As species is its pentavalent state, in the form of adsorbed arsenate (Fe-AsO₄). However, most samples contain a small amount of reduced arsenic, in the form of arsenopyrite (FeAsS), most likely remaining un-altered from the original source (ore).



Figure 13. Arsenic (top) and loss of ignition (bottom) profiles for the Loddon River sites. Profiles extend through the overlying tailings through the original (relic) floodplain (boundary marked by 'contact).

Mining of gold ores in Victoria at the end of the nineteenth century has left a legacy of As-rich tailing deposits in many river catchments of the State, including the Loddon River catchment, as testified by the presence of these shallow As-rich plumes. The weathering and oxidation of arsenopyrite grains may have occurred either during mineral processing, river transport or after river bank deposition. To understand the mechanism, extent and products of the long-term weathering of these tailings and their effect on As cycling, we will investigate the effects of repetitive soil redox cycles, which occur naturally during flooding and draining phases, especially in floodplain environments. Further X-ray absorption spectroscopy studies (extended x-ray absorption fine structure; EXAFS) analysis is proposed in order to better characterize As complexation mechanisms and evaluate its mobility and potential risk for the environment.

James O'Dwyer

Project Title: Flows for fish: Using water flow to promote connectivity, recruitment and genetic diversity for Australian fish species

Due for completion: February 2021

Supervisors: Nick Murphy¹, Katherine Harrisson¹ and Zeb Tonkin²

Affiliations: ¹La Trobe University, Bundoora, Vic; ²Arthur Rylah Institute for Environmental Research, Heidelberg, Vic.

Executive summary: Environmental watering is currently the largest conservation effort within Australia, with over \$13 billion having been invested to enhance ecosystem and native fish health within the Murray–Darling Basin. Despite this, a good understanding of the effects of flow conditions on population processes important for long-term survival (e.g. dispersal and recruitment dynamics) is lacking for many species. Promoting population connectivity and/or genetically diverse offspring from recruitment events provides benefits including reduced inbreeding, greater tolerance to environmental changes, and re-establishment after localised extinctions. To develop a flow regime

which best facilitates dispersal and recruitment, the impact of different flow events on these processes must be quantified.

My project aims to use population genomic information to investigate the current state of population structuring and connectivity in four key Australian fish species, Common galaxias (*Galaxias maculatus* Jenyns, 1842), tupong (*Pseudaphritis urvillii* Valenciennes, 1831), Australian grayling (*Prototroctes maraena* Günther, 1864) and Murray cod (*Maccullochella peelii*). In addition, my research will provide a detailed genomic exploration of breeding and recruitment dynamics of Murray cod populations and their interaction with river flow. Through investigating these population processes, my research helps understand how flows can be managed to best promote genetic diversity within key Australian fish species. Through examining the relationship between river flow and population connectivity, recruitment and breeding, I will provide recommendations for how to facilitate these important population processes.

The objectives of this study are to:

- Determine the population genetic structure and scale of dispersal amongst two native Australian catadromous and one native amphidromous fish species.
- Use a simulation and modelling approach to determine the accuracy of genetic kinship, sibship and parentage estimates under a variety of conditions including variable genetic marker number and uneven sampling design.
- Determine the feasibility of accurate parentage and sibship estimates in species where adult sampling is often difficult and high offspring output is common (as seen in many freshwater fish species.
- Determine the population genetic structure of adult Murray cod in the southern Murray– Darling Basin.
- Examine the genotypes of young-of-year Murray cod cohorts from multiple years to estimate temporal changes in dispersal distance from natal adult populations.
- Determine the relationship between river flow and young-of-year Murray cod dispersal.
- Use genomics analysis of Murray cod larval cohorts to develop fundamental knowledge about the breeding and recruitment dynamics of Murray cod.
- Determine factors such as the relatedness and genetic diversity and dispersal within and between larval cohorts and establish the number of breeding adults across multiple years in two key Murray cod breeding locations, the Goulburn River and the Barmah-Millewa forest.
- Determine the relationship between river flow and breeding success and larval recruitment in Murray cod populations.

Progress to date

 Genetic samples have been sequenced for ninety-four individuals for Common galaxias, tupong and Australian grayling. Analysis of population genetic data has revealed that Common galaxias are connected across the range of the Victorian coastline and represent one large and well-connected metapopulation. Australian grayling and tupong by contrast, appear to display a reduced dispersal capacity and are connected on only more localised scales, with clear west to east structuring being found across the coast of Victoria. Analysis of genetic diversity suggests that tupong and Australian grayling have lower effective population sizes, marking an increased vulnerability to genetic drift if connectivity is not maintained for each species.

- Preliminary analyses examining the accuracy of kinship estimates are providing detailed information regarding the impact of variation in the number of genetic markers, the number of samples and the presence/absence of adult samples. Results so far indicate that highly accurate kinship and sibship estimates can be made without adult sampling using the number of markers typically available through current genomic techniques. Results further indicate that low sample numbers still yield accurate estimates of breeding adult numbers and sibling relationships present in a sample.
- Preliminary analysis of population structuring of adult Murray cod has revealed significant population structure between several of the major rivers within the southern Murray–Darling Basin including the Mitta, Broken, Ovens, Edward, Gunbower and Broken rivers. Approximately 200 young-of-year Murray cod have been collected over three years from 2016–2018 in the Mitta, Broken, Ovens, Goulburn, Campaspe and Murray rivers, with sequencing expected to be completed for all individuals by December 2019.
- Three hundred out of an estimated 460 Murray cod larvae have been collected and DNA extracted from six sites found in the Goulburn River and the Barmah-Millewa forest over four years, with the sampling and sequencing of these samples to be completed by December 2019. Preliminary analyses from 2016 and 2017 in the Goulburn River are revealing key insights into breeding and recruitment within Murray cod. My results estimate the effective population size of the Murray cod across the sampled sites in the Goulburn River is likely to be between 300 and 600, but this number will be refined further as more larvae are sequenced. Between 2016 and 2017, a total of 13 full sibling pairs were found, with the majority of pairs collected from the same sites; however, large movements in the first months (up to 50 km) after hatching have been detected. Preliminary data also suggests that breeding pairs may contribute to multiple clutches of eggs within one season, and additionally may also exhibit within-seasonal polyandry.

Lucas Goncalves Morais

Project Title: The influence of water chemistry on zinc bioavailability and toxicity in Australian freshwaters

Due for completion: November 2021

Supervisors: Aleicia Holland and Ewen Silvester

Affiliations: La Trobe University, Wodonga,

Executive summary: Metal contamination of freshwater ecosystems is a global problem that can be traced back to the Roman Empire and therefore has a close link with human history. Australian freshwaters are no different, with contamination of aquatic ecosystems caused by mining, urbanisation, agriculture and acid sulphide soils. Elevated metal concentrations occur across Australian aquatic ecosystems including parts of the Murray–Darling Basin. Increased metal concentrations can cause toxic effects to aquatic organisms. The toxicity and bioavailability of a metal is not only a function of the total or dissolved metal concentration, but also of a number of key water quality variables, including dissolved organic carbon (DOC), pH and hardness.

The importance of better understanding how environmental variables affect the bioavailability and toxicity of contaminants, such as metals, is well recognized by academia, industry and regulatory agencies — especially with regards to deriving water quality guidelines and environmental risk assessments. Zinc (Zn) is one such metal of concern. Zn is an essential element for many biological functions, with more than 200 enzymes dependent on Zn for normal activity. However, in many

freshwaters, Zn concentrations may be high as a result of anthropogenic activities occurring at levels which are toxic to the aquatic biota. Australian freshwaters vary significantly in their pH, hardness and DOC concentrations, making it often difficult to determine possible risks associated with metals in these systems. Limited knowledge currently exists on the toxicity and bioavailability of Zn to Australian biota under various water chemistries representative of Australian freshwaters. Therefore, the aim of this project is to investigate changes in water chemistry across Australian freshwaters and evaluate what this may mean with regard to the toxicity of Zn, with the ultimate goal of informing future revisions of the Australian and New Zealand freshwater (ANZECC) guidelines.

The first objective of this project was to collate water quality (WQ) data from across Australia to determine median, 10^{th} and 90^{th} percentiles for pH, DOC, and hardness within each state and territory. To date, we have obtained data from Victoria, Queensland, Northern Territory, Western Australia and Tasmania and these values are provided in Table 8. With regard to the Murray–Darling Basin waters, we have so far analysed WQ data for South Australia and these are characterised by median values: pH 7.7 (n= 17,579), hardness CaCO₃ 69 mg.L⁻¹ (n= 2,062) and DOC 4.7 mg.L⁻¹ (n=10,497).

		Median	Percentile 90	Percentile	Number of	Number of
	рН	7.1	8.3	5.6	15,631	1,234
NT	Hardness (mg.L ⁻¹ CaCO3)	55	325	4	16,025	2,349
	DOC (mg.L ⁻¹)	2	14	1	1,332	93
	рН	7.5	8.3	6.6	75,624	383
QLD	Hardness (mg.L ⁻¹ CaCO3)	76	291.9	10	27,681	365
SA	рН	7.7	8.2	7.3	17,579	49
	Hardness (mg.L ⁻¹ CaCO3)	69	109	42	2,062	30
	DOC (mg.L ⁻¹)	4.7	8.6	3.0	10,497	42
	рН	7.1	7.9	6.1	16,473	528
TAS	Hardness (mg.L ⁻¹ CaCO3)	35	290	11	1,394	219
	DOC (mg.L ⁻¹)	14.5	20	10.8	8	1
VIC	pН	7.1	7.7	6.5	412,020	482
	Hardness (mg.L ⁻¹ CaCO3)	38	378.4	11.4	255	111

Table 8. Water quality parameter values for each state.

The water quality parameter, pH, has a very strong effect on Zn speciation and varies significantly across Australia, ranging from 5.6 to 8.3 (10th and 90th percentiles, respectively; Table 1). Given that the toxicity of metals depends on their speciation (with the free ion form Zn²⁺ considered the most toxic), the differences in pH within Australian freshwaters likely have a significant influence on the toxicity and bioavailability of Zn to aquatic organisms. DOC also influences the speciation of metals, and therefore toxicity, by forming complexes with metals such as Zn, converting them from their free ion form to a DOC-metal complex; increases in DOC generally result in a decrease in metal toxicity. Australian freshwaters range widely in DOC concentrations between 1–20 mg.L⁻¹; 10th and 90th percentiles, respectively (Table 1) so DOC concentration is likely to be a major determining factor influencing metal toxicity within Australian aquatic ecosystems. Hardness, which reflects the concentrations of Ca²⁺ and Mg2⁺ present within aquatic ecosystems, also influences the

bioavailability and toxicity of metals through competition with the metal ion for cellular binding sites of aquatic organisms; metal toxicity generally decreases with increasing hardness. Hardness of Australian freshwaters varies greatly and ranges from extremely soft waters (4 mg.L⁻¹ CaCO₃) to very hard (378 mg.L⁻¹ CaCO₃); 10th and 90th percentiles, respectively. H⁺ ions can also compete for binding sites, so pH can also influence toxicity in this manner.



Figure 14. Speciation diagram for the system $Zn^{2+} + H^+ + Cl^- + CO_3^{2-} + SO_4^{2-}$ in a simulated freshwater medium (Powell *et al.* 2015).

The second objective of this project involves exposing the microcrustacean, *Ceriodaphnia dubia*, to Zn^{II} in artificial and natural freshwaters, simulating realistic ranges for: DOC, hardness and/or pH in Australians rivers, to determine how changes in water chemistry will influence Zn^{II} toxicity to this aquatic organism. The applicability of bioavailability models, such as the Biotic Ligand Model (BLM) and Multiple Linear Regression (MLR) models, will also be determined. These models can take into account the influence of physicochemical parameters, such as pH, hardness and DOC, on metal toxicity, speciation and bioavailability, and can be used to derive site-specific guideline values for metals within Australian waters. Predicted toxicity values obtained via use of the BLM or MLR models will be compared to toxicity results obtained within the various test waters. If predicted results fall within \pm a factor of two the models, they will be deemed acceptable for use in Australian waters to determine site-specific guidelines.

Appendix 2: Communications Overview

Introduction

The ultimate goal of the communication section of MMCP is to identify key stakeholders and ways to communicate effectively with them to raise the overall awareness of the project and its outcomes.

A number of activities have been undertaken as part of the overall communication strategy, including informal posts online via social media, to the local community via newspaper articles, scientific community through conferences and seminars as well as via technical and literature review reports.

In addition to the following outputs, the MMCP webpage was launched on the La Trobe University website in May 2017 (<u>https://www.latrobe.edu.au/freshwater-</u><u>ecosystems/research/projects/mmcp).</u>

Publications

Reports

- Baldwin D (2016) The potential impacts of climate change on water quality in the southern Murray– Darling Basin. Final Report prepared for the Murray–Darling basin Authority by The Murray-Darling Freshwater Research Centre, MDFRC Publication 139/2015, December, 59pp. <u>doi.org/10.26181/5d199342d58d5</u>.
- Brown P, Gehrig S (2018) Improving our understanding of the ecosystem effects of varying water levels in weir pools of lowland floodplain rivers: What role can weir pool manipulations play in restoring the health of the River Murray channel? Final Report prepared for the Murray– Darling Basin Authority by The Murray–Darling Freshwater Research Centre, MDFRC Publication 180/2018, April, 19pp. doi.org/10.26181/5d1993d78cf52
- Brown P, Wood D, Dunne C and McKillop T (2019) Fish Movement. Final Report prepared for the Murray–Darling Basin Authority by The Centre for Freshwater Ecosystems, CFE Publication 221/2019, June, 10pp. <u>doi.org/10.26181/5d19866596675</u>
- Haby N, Romanin L, Nielsen D (2019) Can we better mitigate against weed (aquatic and terrestrial) invasions through tailoring flow events in the Murray-Darling Basin? Report prepared for the Murray–Darling Basin Authority by The Centre for Freshwater Ecosystems, CFE Publication 214/2019, June, 19pp. doi.org/10.26181/5d1995c8aa48e
- Huntley S, Brown P (2016) Lock Keeper's guide to PIT tagging fish at Lock 10. Final Report prepared for the Murray–Darling Basin Authority by The Murray–Darling Freshwater Research Centre, MDFRC Publication 112/2016, June, 7pp. <u>doi.org/10.26181/5d199684490c8</u>
- McInerney P, Shackleton M, Rees G, Holland A, Davey C and Petrie P (2019) MMCP Collaboration Final report 2019 - Biofilm succession patterns and ecosystem dynamics in the Edward-Wakool River system. Final Report prepared for the Murray–Darling Basin Authority by The Centre for Freshwater Ecosystems, CFE Publication 219/2019, June, 31pp. <u>doi.org/10.26181/5d19904d570e9</u>

- Mynott JH, Shackleton M, Furlan E, Rees G, Gleeson N and Bond N, (2019) eDNA: review of applicability for monitoring and detecting biotic populations of the MDB. Final Report prepared for the Murray–Darling Basin Authority by The Centre for Freshwater Ecosystems, CFE Publication 213/2019, February, 18pp. <u>doi.org/10.26181/5d1999a3bc14f</u>
- Nielsen D, Baldwin D, Bond N, Brown P, Rees G, Stoffels R, Shackleton M, McInerney P (2016) The MDBA–MDFRC Collaboration Project Annual Report 2015–2016. Report prepared for the Murray–Darling Basin Authority, La Trobe University and CSIRO by The Murray–Darling Freshwater Research Centre, MDFRC Publication 120/2016, July, 3pp.
- Nielsen D, Bond N. (2018) Connectivity and floodplain infrastructure. Report prepared for the Murray-Darling Basin Authority by The Murray-Darling Freshwater Research Centre, MDFRC Publication 179/2018, May, 14pp. <u>doi.org/10.26181/5d199f2cda3cf</u>
- Nielsen D, Durant R (2019) MMCP Collaboration Final report 2019 Vegetation Dispersal. Report prepared for the Murray–Darling Basin Authority by The Centre for Freshwater Ecosystems, CFE Publication 218/2019, June, 32pp. <u>doi.org/10.26181/5d19911fa9947</u>
- Nielsen D, Durant R, Freestone F, (2016) Preliminary report on the dispersal of propagules through pumps. Draft report prepared for the NSW Office of Environment and Heritage by The Murray–Darling Freshwater Research Centre, August, 5pp.
- Nielsen D, Rees G, Brown P, Baldwin, D, Stoffels R, Gawne B, Bond N (2016) The MDBA–MDFRC Collaboration Project – stocktake report. Final Report prepared for the Murray–Darling Basin Authority, MDFRC Publication 137/2016, November, 52pp. <u>doi.org/10.26181/5d199b2ae4ca9</u>
- Nielsen D, Rees G, Brown P, Stoffels R, Bond N (2017) The MDBA–MDFRC Collaboration Project Annual Report 2016–2017. Report prepared for the Murray–Darling Basin Authority, La Trobe University and CSIRO by The Murray–Darling Freshwater Research Centre, MDFRC Publication 149/2017, June, 67pp.
- Nielsen D, Rees G, Brown P, Stoffels R, McInerney P and Durant R (2019) MMCP Collaboration Final report 2019 – Final Report prepared for the Murray–Darling Basin Authority by The Centre for Freshwater Ecosystems, CFE Publication 217/2019, May, 61pp. <u>doi.org/10.26181/5d19927544b20</u>
- Nielsen D, Rees G, Brown P, Stoffels R, McInerney, Durant, R (2018) The MDBA–MDFRC
 Collaboration Project Annual Report 2017–2018. Report prepared for the Murray–Darling
 Basin Authority, La Trobe University and CSIRO by The Murray–Darling Freshwater Research
 Centre, MDFRC Publication 195/2018, June, 30pp.
- Nielsen D, Riddle M (2017) The MDBA–MDFRC Collaboration Project Communication Strategy. Final Report prepared for the Murray–Darling Basin Authority by the Murray–Darling Freshwater Research Centre, MDFRC Publication 149, March, 10pp. <u>doi.org/10.26181/5d199beb32394</u>
- Nielsen DL, Gehrig S, Bond N (2017). How will declining water availability as a consequence of climate change affect habitat and species distributions in the Murray–Darling Basin? Final Report prepared for the Murray–Darling Basin Authority by The Murray–Darling Freshwater Research Centre, MDFRC Publication 170/2017, Sept, 27pp. doi.org/10.26181/5d199c94772e6

- Rees G, McInerney P, Stoffels R, Shackleton M, Nielsen D, Dwyer G, Baldwin D and Silvester E (2019) MMCP Collaboration Final report 2019 – Understanding the ecological consequences of macroinvertebrate community-structure change. Report prepared for the Murray–Darling Basin Authority by The Centre for Freshwater Ecosystems, CFE Publication 220/2019, May, 28pp. doi.org/10.26181/5d198e0d343a6
- Rees GN (2017) Factors contributing to the 2016 hypoxic blackwater event. Report prepared for the Murray–Darling Basin Authority by The Murray–Darling Freshwater Research Centre, MDFRC Publication 158/2017, Sept, 14pp. <u>doi.org/10.26181/5d199d3ce698a</u>
- Stoffels R, Bond N and Nicol S (2016) Science to support the management of riverine flows. Final Report prepared for the Murray–Darling Basin Authority by The Murray–Darling Freshwater Research Centre, MDFRC Publication 138/2016, December, 24pp. <u>doi.org/10.26181/5d1ead54d10d9</u>

Journal publications

Stoffels RJ, Bond NR, and Nicol S (2018). Science to support the management of riverine flows. *Freshwater Biology* **63**(8), 996–1010. <u>doi.org/10.1111/fwb.13061</u>

Completed Theses

- Kidzak S (2017) The ability of PCR-based methods and next generation sequencing to detect secondary predation in the gut contents of aquatic macroinvertebrates. Honours thesis, Department of Ecology, Environment and Evolution, La Trobe University, Wodonga. <u>doi.org/10.26181/5d1ead54d10d9</u>
- O'Dwyer J (2017) Stress tolerance losses from stabilising selection in a salt lake organism. Honours thesis, Department of Ecology, Environment and Evolution, La Trobe University, Bundoora. <u>doi.org/10.26181/5d1ebff03ad1f</u>
- Hill K (2018) Understanding the functional connectivity of non-floodplain wetland plant populations in an agricultural landscape. Honours thesis, Department of Ecology, Environment and Evolution, La Trobe University, Wodonga. <u>doi.org/10.26181/5d1ec13362dc5</u>
- Davis N (2017) The effect of waterhole permanency and hydrological connectivity on fish community composition in a dryland river. Honours thesis, Department of Ecology, Environment and Evolution, La Trobe University, Wodonga. <u>doi.org/10.26181/5d1eb2f1b13ef</u>
- Calipari T (2019) Adverse effects of microplastics observed on the growth rate and health of freshwater alga *Chlorella* sp. 12. Honours thesis, Department of Ecology, Environment and Evolution, La Trobe University, Wodonga. <u>doi.org/10.26181/5d1ec182ca689</u>

Conference Presentations.

Nielsen D (Sep-17) Infrastructure modifies movement of seeds drifting in lowland rivers Australian Society for Limnology. Sydney, NSW. <u>doi.org/10.26181/5d199e8f5b58e</u>

Workshops

Baldwin D (2016) Prediction of Harmful Algal Blooms. HABit Workshop.

- Baldwin D (2016) Presentation to the Murray–Darling Basin Authority Water Quality Advisory Panel, Sydney.
- Nielsen D (2016) Seed bank and wetland dynamics in response to environmental watering. Presentation to the Murray–Darling Basin Authority, Canberra, October.
- Brown P (2016) Fish movement workshop, Ecological objectives of the current and future PIT Tagging program, La Trobe University, Bundoora, Victoria. 4 November.
- Stoffels R (2016) Evaluating fish population dynamic response to flows. Presentation to the Murray– Darling Basin Authority, Canberra. October.
- Brown P, Nielsen D, Stoffels R and Rees G (2017) Overview of MMCP and presentations of data from the four research theme leaders. Presentation to the NSW Department of Primary Industry Seminar Series, Sydney NSW, 28 March.
- Nielsen D (2017) Overview of MMCP Collaboration Project. Presentation to the Department of Agriculture and Water Resources, Canberra ACT. 22 March.
- Nielsen D (2017) Overview of MMCP Collaboration Project. Presentation to the Murray–Darling Basin Authority. Canberra ACT. 21 March.
- Nielsen D (2017) MDBA-MDFRC Collaboration Project and Vegetation dispersal. Presentation to Department for Environment, Water and Natural Resources, Adelaide, SA. 26 May.
- Rees G (2017) Native fish: are they getting the nutrition they need. Presentation to the Murray– Darling Basin Authority, Canberra. May.
- Durant R (2017) Vegetation dispersal. Presentation to the Mallee Catchment Authority, Mildura Vic. 23 May.
- Nielsen D (2017) Vegetation dispersal. Presentation to La Trobe University, Vic. 17 May.
- Rees G (2017) Macroinvertebrate trophic links. Presentation to the Murray–Darling Basin Authority, Canberra. 24 May.
- Nielsen D (2017) Managing wetlands for plants. Presentation to the Floodplain ecology course. Barmah, Victoria. 10 October.
- Nielsen D (2017) Managing wetlands for plants. Presentation to the Brolga round table, Berrigan, NSW. 13 October.
- Nielsen D (2017) Overview of MMCP Collaboration Project. Presentation to the Department of Environment, Land, Water & Planning. Melbourne, Victoria. 27 November.
- Bond N, Nielsen D, Rees G and McInerney P (2018) Overview of MMCP themes. Presentation to the South Australian Department for Environment and Water, Adelaide SA. 27 September.
- Nielsen D, Rees G and Shackleton M (2018) Overview of MMCP themes. North West Victoria and Far South West NSW Engagement Seminar, Mildura Vic. 21 March.
- Nielsen D and Rees G (2019) Results and outcomes of MMCP Vegetation and food web themes. Centre for Freshwater Ecosystems communication engagement seminar, Wodonga Vic. 14 March.

Newsletters and social media.

MDFRC E Newsletter

Seed dispersal surveys in the Mallee	9 October 2017
Collection of decapods from the Ovens River Floodplain	3 December 2017
Welcome new students: Teresa Calipari (Honours)	13 March 2018
La Trobe University School of Life Sciences newsletter	
Lock staff training program	August 2016
CFE E-newsletter	
Fish population theme	August 2018
CSU Internship Student Jenessa Albert completed her 12 week placement	December 2018
PhD top up scholarship opportunity	December 2018
Update on seminar in Mildura	December 2018
Facebook	
Lock staff training	August 2016
Scholarships on offer	24 August 2017
Project Update - Vegetation dispersal	24 October 2017
Project Update – Macroinvertebrates	2 November 2017
Brazilian student to study fish behaviour using specialty flume tank	30 November 2017

brazinan student to study him benaviour using speciality nume tank	50 100 cmber 2017
Matthew hopes for big results after research cadetship (summer scholarship)	22 February 2018
Fish population theme (what is an otolith)	18 June 2018
Post graduate research scholarship	8 August 2018
The movement of seeds (Vegetation theme)	12 September 2018
Farewell to student intern Janessa Albert	9 October 2018
Promoting Mildura seminar	15 November 2018
Promotion of CFE Engagement seminar	14 March 2019
Twitter	
Do regulators modify seed movement?	18 October 2017
What makes yabbies and freshwater prawns healthy	3 November 2017
Brazilian student to study fish behaviour using specialty flume tank	30 November 2017

Postgraduate research scholarship opportunities

October 2018

14 March 2019

Newspaper releases

Sunraysia Dailey	Algae set to hang around	19 May 2016
Border mail	Blooms: are they the new normal?	21 May 2016
Border mail	Cooler temperatures and rain will likely mean the end of the lingeri bloom, says expert	ng algae May 2016
Washington Post	We've primed the system: Why disgusting toxic blue-green algae bl increasingly common	ooms seem 25 June 2016
Conversation	Are toxic algal blooms the new normal for Australia's major rivers?	18 May 2016
ABC	Disruptive Murray River algal blooms to become more common	9 March 2016
EconoTimes	Climate Change Series - Are toxic algal blooms the new normal for A major rivers?	Australia's 18 May 2016
Border mail	Brazilian student to study fish behaviour using specialty flume tank	
	28 N	ovember 2017
Border mail	Matthew hopes for big results after research cadetship 22	February 2018

Fact sheets

Summary and overview

MMCP factsheet #1. Overview of MMCP Collaboration. doi.org/10.26181/5c64f361d67f3

Summary of findings. doi.org/10.26181/5d19a18ad16c8

Education capacity building through postgraduate studies. doi.org/10.26181/5d1acee1d3dd5

Vegetation dispersal

MMCP factsheet #2. Vegetation theme. doi.org/10.26181/5c64f333845b5

Understanding wetland plant seed dispersal and interactions with floodplain infrastructure. <u>doi.org/10.26181/5d1ace2427856</u>

Fish movement

MMCP factsheet #3. Fish movement. doi.org/10.26181/5c64f2fa910d2

Supporting on-going monitoring of fish movement within the River Murray. <u>doi.org/10.26181/5d19a24196a2c</u>

Fish population and community dynamic modelling

MMCP factsheet #4. Impacts of hydrology and climate on the growth dynamics of Murray cod and Golden perch. <u>doi.org/10.26181/5c63a34ad3a6b</u>

How does river flow and temperature affect growth of Murray cod and Golden perch? <u>doi.org/10.26181/5d1ec8e55cb1b</u>

Linking macroinvertebrate community structural changes to ecosystem outcomes.

MMCP factsheet #5. Understanding the ecological consequences of macroinvertebrate communitystructure change. <u>doi.org/10.26181/5c64f2c433c3b</u>

Linking flow variability to food-web and ecosystem function outcomes. doi.org/10.26181/5d19a39e717ea

Response of basal resources to changing flows

MMCP factsheet #6. Response of basal resources to changing flows. doi.org/10.26181/5d199fdd48383

The effects of changing flows on basal resource productivity and quality. <u>doi.org/10.26181/5d1acd798a9a3</u>

Basin Official Committee (BOC) Questions

The potential impacts of climate change on water quality in the southern Murray-Darling Basin. <u>doi.org/10.26181/5d1eaff43be40</u>

How can improved knowledge of the relationships between flows, ecological condition and responses be used to guide environmental water planning and management? doi.org/10.26181/5d1eaf91b823e

Factors contributing to the 2016 hypoxic blackwater event. doi.org/ 10.26181/5d1ad2ac7bddf

How will declining water availability as a consequence of climate change affect habitat and species distributions in the Murray-Darling Basin? <u>doi.org/ 10.26181/5d1acfd2b3e1e</u>

Connectivity and floodplain infrastructure. doi.org/10.26181/5d1ad0d670541

Improving our understanding of the ecosystem effects of varying water levels in weir pools of lowland floodplain rivers: What role can weir pool manipulation play in restoring the health of the Murray River Channel? <u>doi.org/10.26181/5d1ad045a241d</u>

eDNA: review of applicability for monitoring and detecting biotic populations of the Murray-Darling Basin. <u>doi.org/10.26181/5d1ad38d652fe</u>

How can we better mitigate against weed (aquatic and terrestrial) invasions through tailoring flow events in the Murray-Darling Basin? <u>doi.org/10.26181/5d19a2e100a80</u>