



Murray-Darling Basin Environmental Water Knowledge and Research Project

Fish Theme Research Report

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Final Report

CFE Publication 223

June 2019

Murray–Darling Basin Environmental Water Knowledge and Research Project Fish Theme Research Report

Report prepared for the Department of the Environment and Energy, Commonwealth Environmental Water Office by La Trobe University

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Report Citation: Price A, Balcombe S, Humphries P, King A, Zampatti b, (2019) Murray–Darling Basin Environmental Water Knowledge and Research Project — Fish Theme Research Report. Report prepared for the Department of the Environment and Energy, Commonwealth Environmental Water Office by La Trobe University, Centre for Freshwater, CFE Publication 223 June 2019 41p. [Appendices 203p.]

Cover Image: Golden Perch, *Macquaria ambigua ambigua*

Photographer: Gunther Schmida (sourced from the Murray Darling Basin Authority)

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Acknowledgements:

The Murray-Darling Basin Environmental Water Knowledge and Research (MDB EWKR) Project is funded by the Australian Government Commonwealth Environmental Water Office and La Trobe University. The Fish Theme of MDB EWKR is a collaboration between the La Trobe University (LTU), Charles Darwin University (CDU), Charles Sturt University (CSU), Griffith University (GU) and the South Australian Research and Development Institute (SARDI). The Fish Theme is co-ordinated by Amina Price (LTU) with a leadership group comprising Brenton Zampatti (SARDI), Alison King (CDU/LTU), Paul Humphries (CSU) and Stephen Balcombe (GU) providing research direction and oversight. John Koehn (Arthur Rylah Institute), Lee Baumgartner (CSU) and Rick Stoffels (CSIRO) were members of the Leadership Group for the planning and foundational stages of the project. We also gratefully acknowledge Ben Gawne for his leadership and contributions to the Fish Theme.

The Fish Theme benefits from co-investment by the Qld Department of Environment and Science (DES), the NSW Department of Primary Industries – Fisheries and the Victorian Fisheries Authority.

Research that was undertaken in the Northern MDB was supported by Ryan Woods (DES) and Kate Hodges (DES) who provided input into the selection of field sites and study design. Kate Hodges also assisted with all field work and Priya Muhid (DES) quantified and identified the algae and zooplankton. DES also provided laboratory support for the dietary analysis.

Golden Perch and Murray Cod larvae were supplied by the NSW Department of Primary Industries Fisheries hatchery at Narrandera and the Uarah Hatchery, also at Narrandera and the Victorian Fisheries Authority's hatchery at Snob's Creek. Thank-you to Matt McLellan NSW DPI Fisheries for additional advice and assistance with larvae.

Golden Perch and Murray Cod larval experiments were undertaken by Amina Price, Kyle Weatherman (LTU) and Matthew Brewer (LTU student) and Paul Humphries and Rick Stoffels provided advice regarding experimental design.

Field work in the Ovens River Floodplain was undertaken by Paul Humphries, Amina Price, Rochelle Petrie (LTU), Luke McPhan (CSU), Stacey Kopf (CSU), Kyle Weatherman (LTU), Chris Davey (LTU), Keller Kopf (CSU), Robert Cook (CSU), Lauren Sheather (CSU), Kel Thomas, Jarrod Chapman, (CSU) and Dale Campbell (CSU). Laboratory processing of samples was done by Rochelle Petrie, Garth Watson, Kyle Weatherman, Jarrod Chapman, (CSU) and Dale Campbell (CSU). Data analyses were conducted by Amina Price, Paul Humphries and Luke McPhan.

Nicole McCasker, Keller Kopf, Amina Price and Rick Stoffels contributed to development and design of the main channel component of the Ovens Floodplain project. Geoff Vietz and Mick Donges collaborated and advised on the flow retention and geomorphic complexity part of the project. Rick Stoffels also contributed to the development and planning of the floodplain component of the Ovens River work.

Stacey Kopf contributed to development, design, execution of field work and writing up of the larval release project. Nicole McCasker, Keller Kopf, Amina Price, Alison King, Brenton Zampatti and Harry Balcombe contributed to development and design of the project. Thanks to Brent Womersley, Anthony Forster, Jane Plischka, John Douglas and Brett Ingram from Victorian Fisheries for assistance with logistics and permits. Liz Dalglish-Wright, Parks Victoria, for assistance with permits.

Research activity 7 involved a large amount of collaboration across agencies for sampling, research support and funding. Water $^{87}\text{Sr}/^{86}\text{Sr}$ analysis was undertaken by Roland Maas at the University of Melbourne. Water sample collection for $^{87}\text{Sr}/^{86}\text{Sr}$ analysis was made possible through funding from the Commonwealth Environmental Water Office (CEWO), the Murray-Darling Basin Authority (MDBA), the multi-jurisdictional MDB Joint Venture Monitoring and Evaluation program, and a NSW Recreational Fishing Trust Grant. Golden Perch sampling across the MDB was funded by the MDBA, the CEWO LTIM and EWKR programs, and a range of State based monitoring programs in NSW, Victoria and Queensland. Murray Cod otoliths were generously provided by NSW Fisheries, the Victorian Department of Environment, Land, Water and Planning, and the South Australian Research and Development Institute. Otolith $^{87}\text{Sr}/^{86}\text{Sr}$ analysis was undertaken by Aleksey Sadekov and Peter Scott at the University of Western Australia. The EWKR Fish Theme Leadership team provided constructive review at pertinent stages of the project.

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Executive Summary

Key outcomes

- Fish recruitment responses vary because of differences in food, habitat and the requirement and capacity for movement or retention of young fish. These are largely governed by differences among life-history strategies and vary among species (2.3).
- The spatial scale at which fish population dynamics occur varies from small (e.g. patch to site) to very large (catchment to Basin) and varies among species. For example, population dynamics of Murray cod operate at scales of 10s–100s kms, while Golden perch population dynamics operate at larger spatial scales again, 100s–1000s kms (2.9).
- Environmental water management that aims to promote Golden perch population growth should consider the large spatial scale in which it operates, and the need for hydrological and physical connectivity for all life stages. Water management outcomes for this species should also be viewed at this scale.
- Hydrological and hydraulic diversity and connectivity at reach to basin scales are critical for maintaining populations at large spatial scales (2.9).
- Smaller scale (patch, site, reach, river segment) processes such as food and larval retention, larval dispersal and temperature regimes create appropriate conditions for growth and survival of individuals and contribute to the diversity of features at nested larger scales.
- Maintaining a diversity of hydraulic, structural and geomorphic conditions in river reaches will allow fish larvae to either be retained in, or to move through reaches depending on their suitability.
- At patch scales, the survival and growth of larvae is strongly related to the interaction between food density and temperature (2.4), with (in general) more food being required to sustain larvae with higher water temperatures.
- Managing the timing of flows to maximise optimal temperatures for early-life stages, are likely to strongly impact on recruitment success. Implications of cold-water pollution on early life stages should also be more strongly considered.
- Food densities for fish early life-stages is related to water retention time. Within the main channel of rivers, flow interacts with the structural and geomorphic complexity within a reach to influence water retention time (2.5 and 2.6). Anabranch habitats were also found to contain high density larval food (zooplankton).
- Access to high food densities may also be managed by providing connectivity to, and from, inundated anabranches and floodplain wetlands, and in the main channel increasing physical complexity of a reach or altering flows to increase retentiveness.
- Downstream drift of early-life stages is not completely passive, even for poor swimming species. Flow retention and discharge influence the dispersal and/or retention of larvae through a reach such that more larvae can be retained in hydraulically, structurally and geomorphically complex reaches (2.8).
- During dry or drought conditions, refuges play a critical role in sustaining populations. There is a need to appropriately manage these areas, using both flow and non-flow related measures, to provide resilience over the longer term (2.5)
- A key outcome of the EWKR fish theme research is the importance of hydraulic diversity, and hydrological/physical connectivity; and the importance of incorporating these concepts into both flow and non-flow management interventions from patch to catchment scales for promoting successful fish recruitment.
- Fish recruitment success is related to many drivers, of which flow is only one. For example, physical and hydraulic complexity, food and temperature suitability and dispersal ability are other key drivers of recruitment for riverine fishes. These non-flow drivers, and their interaction with flow, need to be more fully considered in management interventions and future research.

The distribution and abundance of native fish species within the Murray-Darling Basin (MDB) has declined significantly in the last 50–100 years (Koehn & Lintermans 2012; MDBC 2003). As such, restoring native fish populations is now a key goal of several MDB basin-wide environment programs, including The Basin Plan.

The Basin-wide Environmental Watering Strategy (BEWS) lists improvements in distribution, abundance, population structure and movement as expected outcomes for fish (MDBC 2003). However, to appropriately design environmental watering programs to benefit native fish, links between key watering parameters and potential fish responses need to be clearly understood. This requires knowledge of the processes that maintain fish populations, the key drivers of these and their interaction with flows.

The MDB EWKR Fish Theme focussed on fish recruitment and sought to improve our understanding of the key drivers, functional processes and limitations of successful recruitment of native fish. This would lead to an improved capacity to predict fish recruitment outcomes in response to different environmental water parameters.

At the commencement of the EWKR project, there was a good broad-scale conceptual understanding regarding the key drivers of fish recruitment in the MDB. However, our ability to manage environmental flows to enhance fish recruitment was limited by a lack of knowledge regarding:

1. the relative importance of individual drivers
2. the interactions among recruitment drivers, flow and non-flow related stressors and threats; and
3. how these relationships vary among species.

The Fish theme sought to improve our understanding in relation to these limitations and importantly, tried to do so in a scale-explicit context so that water managers can utilise research outcomes and undertake management actions at the most appropriate scales.

Prior to the development of the research plan, three key foundational activities were undertaken:

1. Identification and assessment of the relative influence of non-flow related factors on key fish recruitment drivers (see section 2.1).
2. Review of current knowledge and management needs on fish recruitment within the MDB (see section 2.2).
3. Development of a conceptual framework for fish recruitment in rivers (see section 2.3).

A key outcome from the conceptual framework (section 2.3), was that recruitment was determined by the spatial and temporal coincidence of optimal conditions (high food, low predation, optimal temperature) with the presence of larvae (movement and retention). Based on this and other elements of the three foundational activities, a research plan was developed that addressed three focal key questions:

1. What are the food and temperature requirements of the early-life stages of target species?
2. Where in the riverscape, and under what flow conditions, are these food and temperature requirements best met?
3. How do dispersal and retention influence recruitment?

In summary, our research demonstrates that both fish population processes, and the key drivers that support recruitment operate at multiple spatial scales, from the patch to the basin scale. Food production areas coupled with appropriate temperature regimes, and the ability for larval dispersal or retention at patch to segment scales, were found to be important drivers of recruitment success. Hydraulic diversity is integral to providing appropriate conditions for food production and dispersal/retention of early-life stages. Connectivity at small to large scales is also critical in enabling access to appropriate habitats and dispersal to maintain populations at catchment to basin scales. Our research also demonstrated that under drought conditions, additional factors such as abundance and condition of adults, and maintenance of refuge areas are likely to be playing a critical role in determining recruitment success.

1 Introduction

The distribution and abundance of native fish species within the Murray-Darling Basin (MDB) is thought to have declined significantly in the last 50–100 years (Koehn & Lintermans 2012; MDBC 2003). As such, restoring native fish populations have become a key goal of a several MDB basin-wide environment restoration programs, including The Basin Plan. The Basin-wide Environmental Watering Strategy lists improvements in distribution, abundances, population structure and movement as expected outcomes for fish (MDBC 2003). However, to appropriately design environmental watering programs to benefit native fish, links between key watering parameters or levers (such as the timing of water delivery, appropriate places to water) and potential fish responses need to be clearly understood. This requires detailed knowledge of the processes that maintain MDB fish populations, the key drivers of these and their interaction with flows. Improving this knowledge gap was the focus of the Fish Theme in the EWKR program, with research addressing: (i) how processes and drivers are influenced by flow; (ii) how this varies based on underlying species traits; and (iii) the spatio-temporal variability in these relationships.

1.1 Scope

The overarching question asked by the MDB EWKR Fish Theme was:

What are the drivers of sustainable populations and diverse communities of native fish?

This question underpinned the Fish Theme research activities, and research was directed to explore the key functional processes that drive outcomes for native fish populations and communities, as well as the conditions under which each of these processes become limiting.

This high-level question was broken down into three priority areas:

1. recruitment of native fish populations (highest priority)
2. survival and condition of native fish populations (medium priority)
3. reproduction of native fish populations (lowest priority).

Specific questions for each priority area were:

- What flow regimes best support the reproduction/recruitment/condition and survival of native fish populations?
 - How significant are the individual drivers?
 - How do key drivers interact to influence outcomes?
 - Under what conditions do these individual drivers influence outcomes?
 - How should flows be managed to enhance drivers and thereby the fish response?
- How do threats impact on the drivers and reproduction outcomes?

The three priority areas encompass the entire life-cycle for fish and therefore all potential processes and drivers. The Fish Theme Leadership Group decided that attempting to undertake targeted work for all priority areas would result in resources being spread too thinly; and therefore, decided to focus on the first priority area: recruitment of native fish populations.

1.2 Approach

The Fish Theme sought to improve our understanding of the key drivers and functional processes of successful recruitment of native fish in the MDB, as well as the situations under which key processes become limiting. This then leads to improved capacity to predict fish recruitment outcomes in response to different environmental flow conditions.

We defined recruitment as the number of 0+ (new) individuals entering the population each year, as measured at a time following hatching, such that individuals have passed through the period when most larval mortality has occurred (Humphries et al., in press).

Recruitment Drivers

At the commencement of the EWKR project, there was a good broad-scale conceptual understanding from the scientific literature regarding the key drivers of fish recruitment (Figure 1). It was well understood that fish recruitment is dependent on three key elements:

1. Spawning magnitude which is influenced by the size and condition of adult populations
2. Appropriate spawning cues and habitat for early life stages
3. Mortality rates of all pre-adult life stages.

Mortality rates for fish are typically very high in the early-life stages, with changes in mortality rates during the embryonic and larval stages potentially leading to changes in juvenile abundances and subsequent recruitment levels (Houde 1987). Mortality can be caused by both intrinsic (morphological or physiological faults) and extrinsic factors (King *et al.* 2013). Extrinsic sources of mortality (external to the individual) are most commonly linked to starvation, predation, disease and other sources of mortality such as environmental stress (King *et al.* 2013).

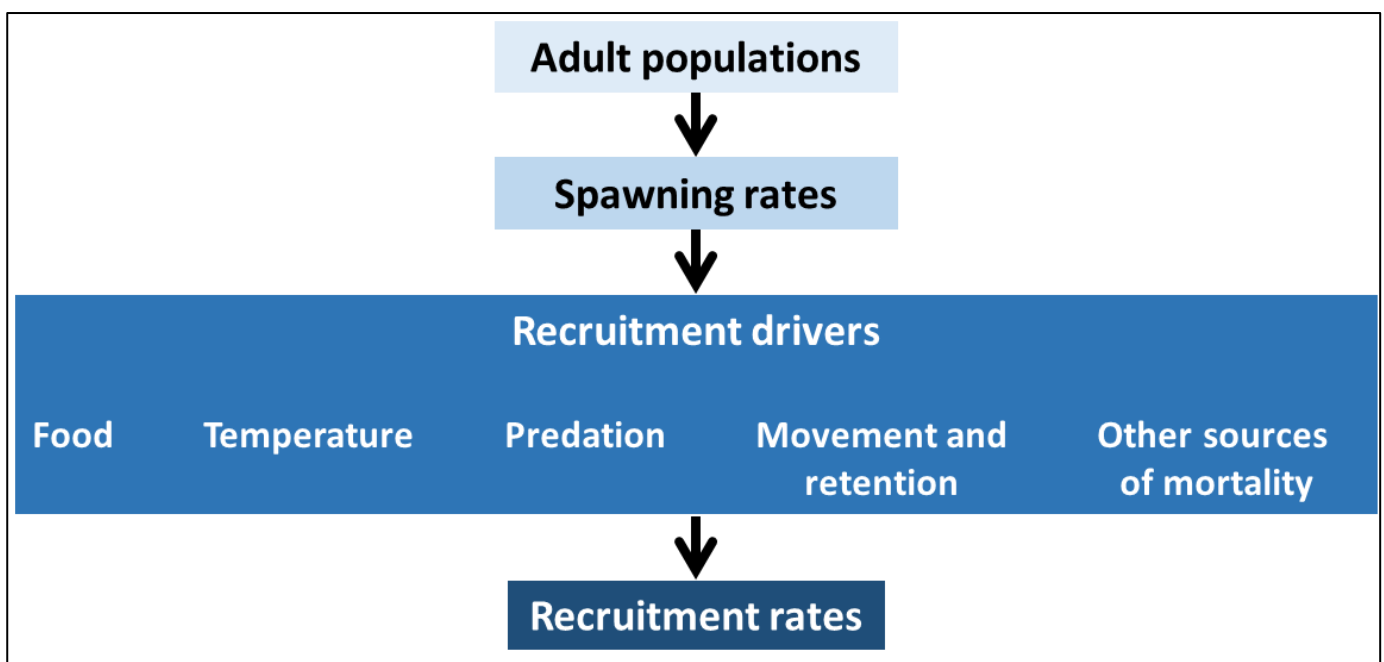


Figure 1. Conceptual model depicting key drivers of fish recruitment.

Whilst this information provides us with a basic understanding of the drivers that underpin fish recruitment, our ability to use this information to manage environmental flows to enhance fish recruitment responses is limited by a lack of knowledge regarding:

- the relative importance of individual drivers
- the interactions among recruitment drivers, flow and non-flow related stressors and threats; and
- how these relationships vary among species.

The Fish Theme sought to improve our understanding in relation to these limitations and importantly, tried to do so in a scale-explicit context.

Incorporating scale

Rivers and streams are complex ecological systems, with different biological and ecological processes operating and interacting with each other at variable scales ranging from millimetres to hundreds of kilometres, and from individual organisms to ecosystems (Figure 2) (Fausch *et al.* 2002). For example, individual survival of young fish is dependent on the immediate food, temperature and water quality conditions and predation pressure at patch to reach scales. At the population scale the dynamics of some

species may be operating at a catchment to Basin scale (Zampatti & Leigh 2013) suggesting that connectivity and movement may be key drivers of recruitment at this scale.

Typically, scientific research within the MDB is based on work that is undertaken at the site to reach scales (Koehn *et al.* 2019), with little or no linkage to processes that occur at smaller (patch) or larger (segment to Basin) scales. Consequently, the results from these studies do not enable an integrated understanding of the functioning and relative importance of drivers and processes at different spatial scales. This limits the ability of water managers to utilise research outcomes and undertake management actions at the most appropriate scales. Incorporation of operating scale was a key consideration in our research focus.

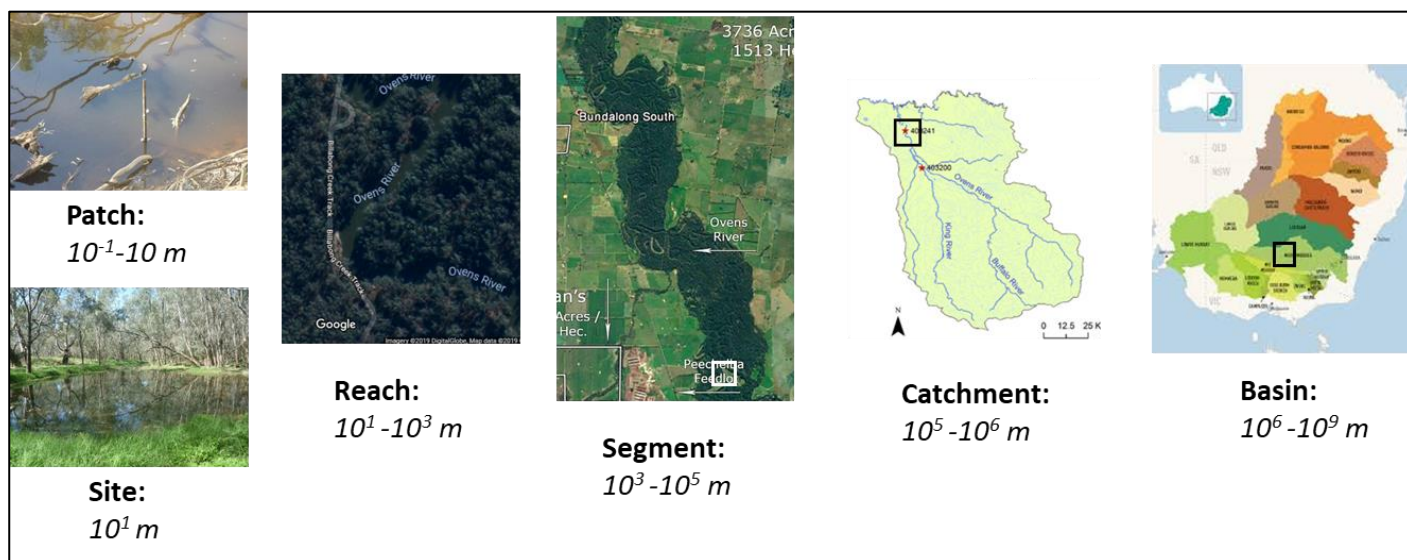


Figure 2. Spatial scales, based on Fausch *et al.* (2002), applied to the Ovens River as an example.

Development of research plan and key research questions

In developing a research plan for the Fish Theme, key requirements were that:

- causal processes and mechanisms were able to be identified;
- the research be based on a testable conceptual framework;
- the role of critical non-flow related threats and stressors be investigated;
- that drivers, processes and responses are considered within a multi-scale context; and
- the research provided outputs that had clear management relevance.

To achieve this, three key foundational activities were undertaken prior to the development of the research plan:

1. A review, synthesis and assessment of the relative importance of non-flow related threats and stressors on key fish recruitment drivers (see section 2.1).
2. A review of the current knowledge and management needs of fish recruitment within the MDB. This included consultation with managers regarding knowledge gaps and priority species (see section 2.2).
3. The development of a conceptual framework for fish recruitment in rivers (see section 2.3). This included reviewing existing fish (both marine and freshwater) and riverine functional models in the scientific literature.

Based on the foundational work (section 2.1 – 2.3), recruitment is thought of as being determined by the spatial and temporal coincidence of optimal conditions (high *food*, low *predation*, optimal *temperature*) with the presence of larvae (*movement and retention*).

Two focal species, Murray Cod (*Maccullochella peelii*) and Golden Perch (*Macquaria ambigua*), were chosen based on the management priorities that were identified (section 2.2).

Based on the outputs of the three foundational activities, a research plan was developed that addressed three key questions at the relevant spatial scales:

Focus area 1: What are the food and temperature requirements of the early-life stages of target species?

This question relates to conditions at the patch-scale and was investigated experimentally using Murray Cod and Golden Perch larvae (see section 2.4).

Focus area 2: Where in the riverscape, and under what flow conditions, are these food and temperature requirements best met?

This question relates to temperature conditions and food available at the site, reach and segment scales, and was approached with three field-based projects. One examined temperature, food and recruitment within individual waterholes (see section 2.5); the second investigated temperature regimes and food availability across different floodplain habitats (see section 2.6); the third examined food production within the main channel in relation to flow, the physical complexity, and the associated retentiveness of different river reaches within a river segment (see section 2.7 and 2.8).

Focus area 3: How do dispersal and retention influence recruitment?

This question relates to the movement of young fish at reach, catchment and basin scales and their capacity to be retained within appropriate habitats and was approached using two field-based studies. The first involved the release of Golden Perch and Murray Cod into reaches with different levels of retentiveness at different flows (see section 2.8). The second examined recruitment patterns of Golden Perch and Murray Cod at catchment to basin scales and how these patterns relate to flow (see section 2.9).

2 Individual Research Activity Summaries

2.1 The influence of flow and non-flow related stressors on the drivers of fish recruitment

Amina Price, Ben Gawne

2.1.1 Synthesis Aims and Objectives

The capacity for fish to respond positively to flow management activities is dependent on a variety of factors, some of which may not be directly related to flow. These include stressors that are associated with flow regulation, for example floodplain disconnection, irrigation extraction, barriers to movement and thermal regimes, as well as others that may not be flow-related such as habitat destruction/degradation, over-exploitation and the introduction of alien species (MDB, 2004). These, in combination with changes to the flow regime, can significantly impact the key drivers and processes that underpin fish recruitment responses.

This foundational activity synthesised existing knowledge to address the following questions:

- What are the key drivers of fish recruitment?
- What is our current evidence for the level of impact that flow versus non-flow related stressors are having on MDB fish species?
- What is the relationship between these drivers, flow and other stressors?
- What is the relative influence of flow versus non-flow related stressors?
- Are complementary actions needed where factors can't be influenced with flow?

2.1.2 Synthesis Outcomes Summary

Key drivers of fish recruitment are defined here as those factors which are direct causes of mortality and sub-optimal growth. These include the quality and quantity of foods ingested; water temperature; predation; disease and parasites; water quality; pollutants and other sources of mortality (e.g. infrastructure). In addition, movement and retention were included because of the critical importance of these processes in enabling fish to access and to be retained within suitable habitat.

The relationship between recruitment drivers, mediating drivers (e.g. nutrient and carbon inputs, hydraulic habitat, connectivity), key threats and associated impacts, and the hypothesised strength of these relationships were depicted using conceptual models (an example is shown in Figure 3).

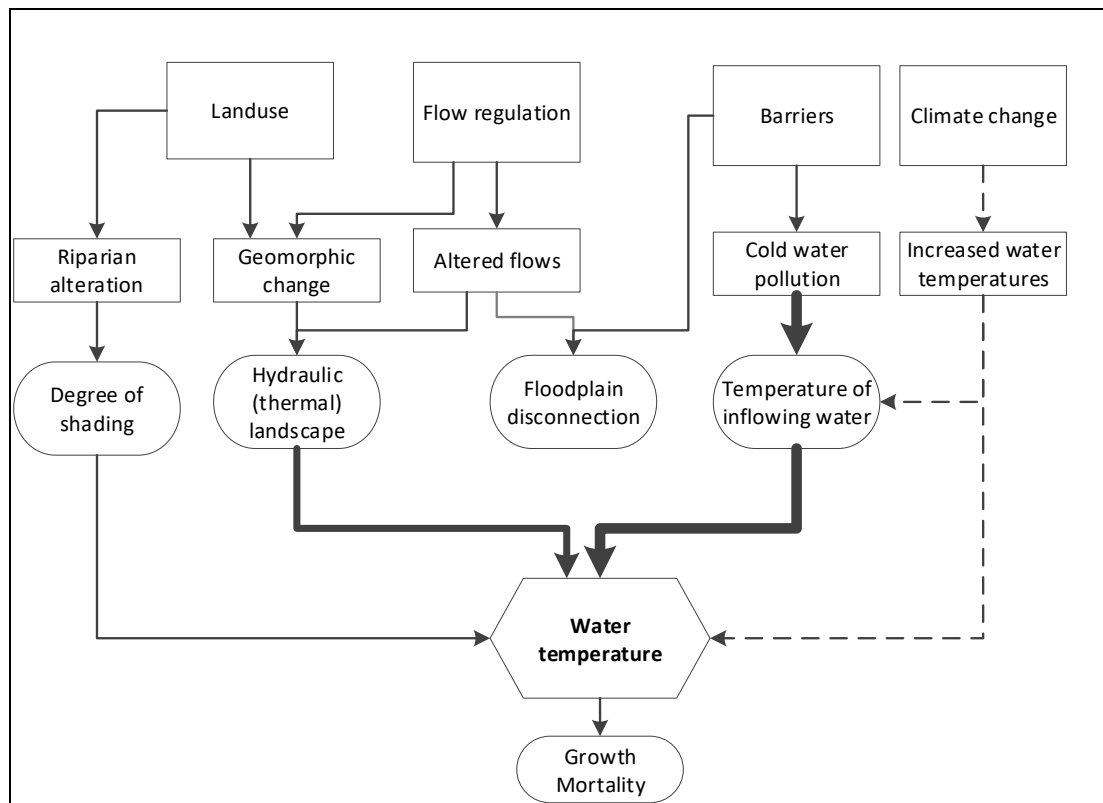


Figure 3. An example conceptual model showing the relationship between key threats and stressors (rectangles), mediating drivers (ovals) on the key recruitment driver, temperature in the main river channel. The width of the lines depicts the strength of the relationship. Where the strength of the relationship is uncertain, the line is dashed. Further conceptual models for other mediating and direct drivers, and system types are provided in Appendices.

A systematic literature review was undertaken to determine the degree of evidence for the impact of flow and non-flow related stressors on mediating and direct drivers of fish recruitment. This review showed that within Australia, the impacts of flow are relatively well-studied, however, the number of published studies examining other stressors or drivers are low (Figure 4).

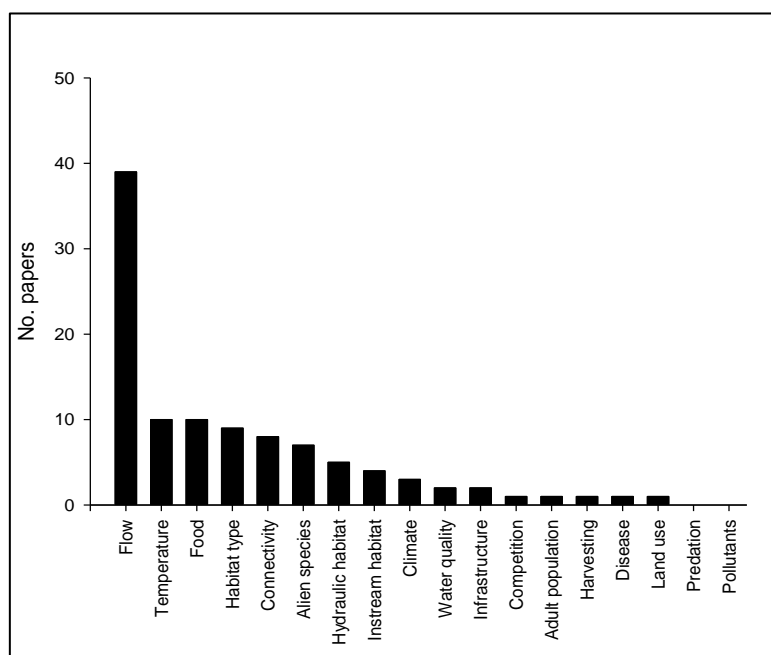


Figure 4. Number of Australian published papers (1991-2019) by direct recruitment driver or mediating recruitment driver.

Based on both the conceptual modelling and the literature review, the key outcomes can be summarised as follows:

- There is evidence that flow regulation impacts on recruitment, however the mechanisms underlying this are not well understood.
- There is good evidence of the impact of temperature on spawning. However, there is limited information regarding its effect on survival and growth. There is little information regarding optimal, sub-lethal and lethal temperature ranges for eggs, early-life-stages and juveniles.
- Food quantity and quality are likely to be key drivers of recruitment; however, we understand little of the relationship between prey type and density and rates of growth and survival of larvae and juveniles. The processes and drivers underlying food production are not clearly understood but are likely to be strongly impacted by flow, land use change, altered connectivity, habitat degradation and changes in water quality.
- The effect of predation and competition on recruitment rates is unknown. However, it is likely that rates may be significantly affected by habitat degradation and alien species.
- There is limited information on water quality tolerances of early-life stages of MDB fish species.
- There is evidence for increased mortality rates associated with barriers and infrastructure, particularly of drifting eggs and larvae. Recruitment rates may also be affected by decreased capacity for movement and stranding on floodplains. However, the implications and strength of association on recruitment success have not been investigated.

2.1.3 Water Management Application

Our synthesis has demonstrated that most recruitment drivers are more strongly influenced by non-flow related factors such as land use impacts (e.g. sedimentation, riparian alteration) and barriers than they are by flow alone. Consequently, our capacity to improve fish recruitment outcomes by focussing on flow management alone is probably low. Complimentary actions that may improve recruitment outcomes include:

- Riparian management
- Revegetation
- Removals of barriers and levee banks
- Mitigation of cold-water pollution
- Management of non-native species
- Management of instream habitat

Of all the recruitment drivers considered, food quality and quantity, and movement and retention were the drivers most likely to be directly affected by flow (Figure 5); although the nature of the relationship between flow and these factors is poorly studied.

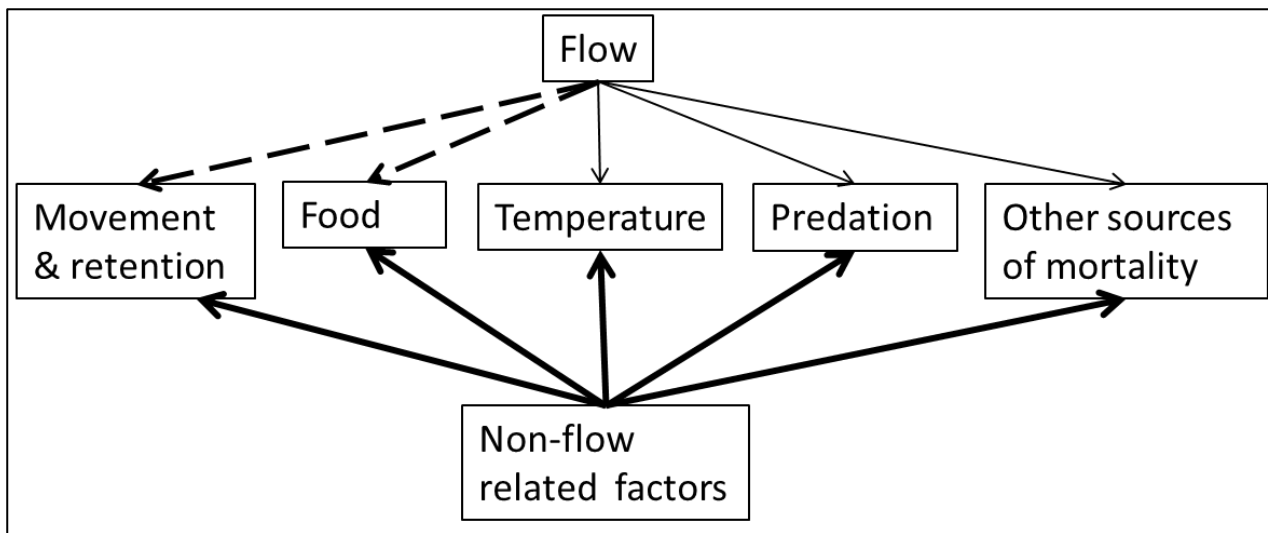


Figure 5. Conceptual model depicting the relative importance (depicted by thickness of lines) of flow versus non-flow related factors on the direct drivers of fish recruitment. Dashed lines indicate that there are significant knowledge gaps regarding the nature of the relationship.

2.2 Prioritising fish research for flow management in the Murray-Darling Basin

John Koehn, Stephen Balcombe, Brenton Zampatti

2.2.1 Synthesis Aims and Objectives

Effective natural resource management requires knowledge exchange between researchers and decision makers to support evidence-based decision making. To achieve this there is a need to ensure research is aligned with current management, policy and subsequent on-ground management actions. The lifecycles of fishes are inextricably linked to flow, and in the MDB, flow management is considered fundamental to the restoration of fish populations. This project set out to identify knowledge gaps regarding the flow-related ecology of freshwater fish to inform research that better informs environmental water management. We reviewed contemporary scientific knowledge and the knowledge base and needs of water managers. Our major objective was to provide an up-to-date synthesis of knowledge pertaining to the flow-related ecology of MDB fishes, from both scientific and management perspectives, and use this to guide fish research for this MDB-Environmental Water Knowledge and Research (EWKR) project.

2.2.2 Synthesis Outcomes Summary

- A workshop between managers and fish ecologists revealed general agreement between groups on what is needed to better manage fish populations using environmental flows.
- Managers want to see “improvements to native fish populations” – observed through greater abundance of adults.
- Managers key species of concern are large-bodied, iconic species, especially angling species such as Murray Cod and Golden Perch; but they are also focussed on small-bodied species of conservation concern (e.g. southern purple-spotted gudgeon (*Mogurnda adspersa*)).
- Managers are looking for more information to guide and support their environmental watering actions and to be able to share this applied knowledge with stakeholders.
- Managers desired a change of spatial and temporal focus of research from limited site and single watering events, to scales that could include connectivity linking multiple events and catchments.
- Managers wanted more understanding of the confidence in transferring results from one region or catchment to others, especially from southern to northern regions of the MDB.
- Synthesis of the current state of MDB fish-flow ecology science has grown rapidly in the last decade but is still narrowly focussed on a few large-bodied species in relation to recruitment ecology (e.g. Golden Perch, Murray Cod and black bream (*Acanthopagrus butcheri*)).
- Scientific knowledge is still limited for relative survival and growth rates between fish life-stages, and how these are influenced by flow.
- Managers want to develop stronger collaborative relationships with their scientific counterparts.
- Both managers and ecologists recognise a need for closer relationships built on trust to ensure common understanding and language that will better support flow management outcomes for fish.

2.2.3 Water Management Application

- Effective water management for fish outcomes needs to be based on the best available science. This occurs when there are strong relationships between managers and scientists; ensuring efficient two-way knowledge transfer.
- The key knowledge gaps for successful environmental flow management need to be appreciated and filled where possible.
- The main identified knowledge gap for most native MDB fish was the influence of flow on survival rates between each life-stage; and ultimately on population growth rates.
- This supports the research focus on fish recruitment of EWKR fish theme.

This output is published as:

Koehn JD, Balcombe SR, Zampatti BP (2019) Fish and flow management in the Murray–Darling Basin: Directions for research. *Ecological Management & Restoration* **20**, 142-150.

2.3 Riverscape recruitment: a conceptual synthesis of drivers of fish recruitment in rivers

Paul Humphries, Alison King, Nicole McCasker, R. Keller Kopf, Rick Stoffels, Brenton Zampatti, Amina Price

2.3.1 Synthesis Aims and Objectives

For a young fish to develop, survive and recruit successfully, they need to be in an area of suitable water quality, be able to find enough food and avoid being eaten. Importantly, the life history and biological traits of the offspring of different species (e.g. egg and larval size, number and dispersal capability) will influence the interactions with these drivers (Winemiller & Rose 1992).

Most fish recruitment models (mostly of marine origin) consider only one or a few drivers in isolation, rarely include species' traits and have limited relevance to riverine environments. Despite their diversity, riverine fishes share enough characteristics to enable predictions of recruitment. This project aimed to review the literature and synthesize the essential components of fish recruitment hypotheses and the key river features, and then develop a model that predicts relative recruitment strength, for all fishes, in rivers under all flow conditions.

2.3.2 Synthesis Outcomes Summary

- The Riverscape Recruitment Synthesis model (Figure 6) combines key discharge-related environmental factors (e.g. energy, nutrient concentration, temperature), fish species traits (e.g. life history) and levels of food and predation to predict recruitment strength of riverine fish.
- It proposes that interactions between flow and physical (i.e. hydraulic, geomorphic and structural) complexity will create locations in rivers where energy and nutrients are enriched, the production of zooplankton prey concentrated, and prey and fish larvae located so that the fish larvae can feed, grow and recruit.
- Reaches in rivers that are ideal for producing and retaining food for fish larvae are ones where high physical complexity translates to high flow retention. Physical complexity creates a mosaic of habitat patches where high levels of nutrients are concentrated, algae grow, which is grazed by zooplankton – a key food source for the young stages of fish.
- Fish larvae and their prey are generally poor swimmers, and as such, flow retention provides the opportunity for both to be in the same place.
- The importance of physical complexity and flow retention will likely change with discharge, and be maximized at intermediate, in-channel discharge and minimum at the lowest and highest discharges.
- Physical complexity and the resulting high flow retention are insufficient to guarantee successful recruitment, however, because other effects, such as lack of sheltering habitat and poor hydraulic diversity and the presence of predators and alien species can overwhelm the positive effects. There is a need for a diversity of flows, from low-flows to flooding, and a diversity of hydraulic and physical complexity, to promote recruitment for riverine fish assemblages.
- Whereas previously the focus of adding or enhancing structure in rivers for fish has been on providing adult habitat, the synthesis model provides a solid rationale for why physical complexity is important for fish early life stages.
- Environmental flows are often designed to enhance riverine fish populations in flow-altered rivers. The Riverscape Recruitment Synthesis model can be used to explore flow and non-flow elements of a river (e.g. temperature, physical complexity, flow) that could be manipulated by managers in all river types.
- The model can be used to make predictions of recruitment strength and can readily be used to test which species are likely to benefit and which could be impaired by flow alteration, based on life history and knowledge of the physical complexity of river reaches.

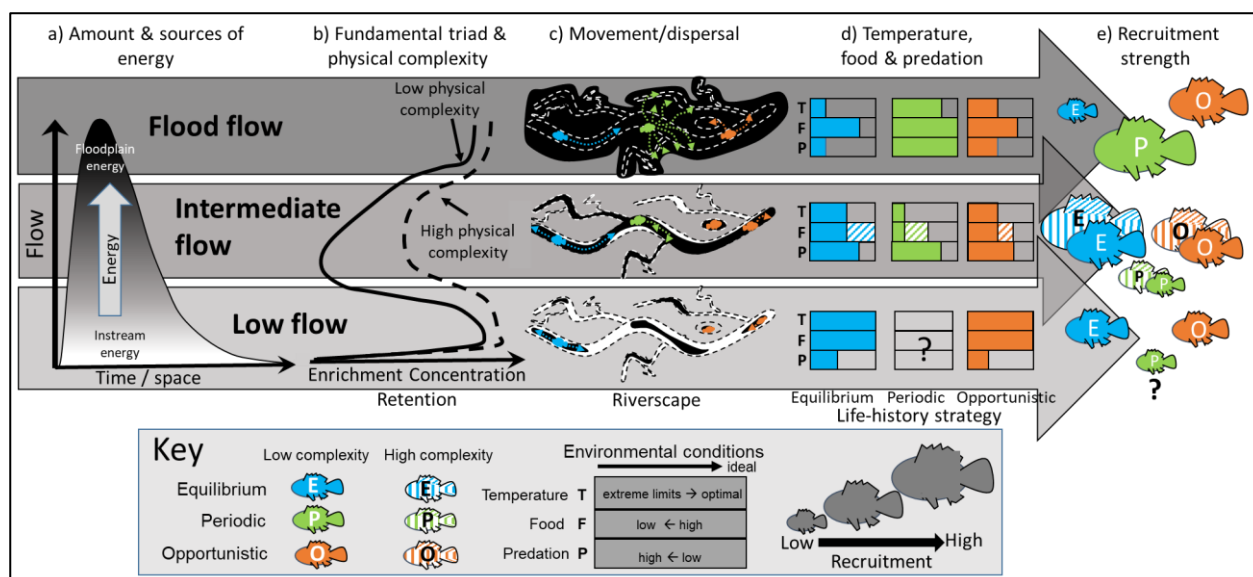


Figure 6. Riverscape Recruitment Synthesis model. The relationship between flow and: a) the amount & sources of energy in rivers; b) the Fundamental Triad mediated by physical complexity of the river reach; c) the potential for movement and dispersal of eggs & young fish (note: no periodic species are included during low flow); and how this combines with d) hypothesized temperature (Temp. & T), food (F) and predation (P) levels considered relevant for fish from opportunistic (blue), periodic (green) and equilibrium (orange) life history strategies; to predict e) relative recruitment strength for fish in low complexity (solid fish) and high complexity (hatched fish) reaches. For b), solid line = low physical complexity, dashed line = high physical complexity. ? = uncertainty of breeding.

2.3.3 Water Management Application

- Recruitment strength can be predicted using this model for all species in all types of rivers based on a few of variables; discharge, reach physical complexity, temperature and food availability. It is also necessary to identify the life history strategy (opportunistic, periodic, equilibrium) of the target species. Predation is likely to be an important factor too but would be much harder to quantify. Thermal optima for processes during egg and larval development can be related to historic or modelled temperature patterns. It would be possible to construct a mathematical model, based on this conceptual one, to predict recruitment quantitatively.
- Establishing flow-complexity-retention relationships is essential for understanding the potential of river reaches to support recruitment of native (and non-native) fishes.
- Flow-complexity-retention relationships are likely to be reach-specific and so the complexities of interactions of flow and reach morphology needs to be considered in any management actions.
- Manipulating flows could be accomplished during peak spawning times to maximise retention capacity of the main stem of rivers, while ensuring structural, hydraulic and geomorphic diversity. Generally, this should satisfy the broad spectrum of species.

This output is published as:

Humphries P., AJ King, N McCasker, RK Kopf, R Stoffels, B Zampatti, and A Price (In Press). Riverscape recruitment: a conceptual synthesis of drivers of fish recruitment in rivers. *Canadian Journal of Fisheries and Aquatic Science*.

2.4 Examination of the relationship between food density, temperature and fish early-life stage growth and survival

Amina Price, Kyle Weatherman, Paul Humphries, Matthew Brewer

2.4.1 Research Question

Starvation and predation are thought to be the primary causes of mortality for the early life stages of fish (Houde 2002). The risk of both predation and starvation generally declines with increased size and development, and consequently rapid growth is vital to reducing mortality risk during the early life-stages (Jones 2002; Trippel *et al.* 1997; Werner 2002). Growth rates are influenced by a variety of biotic (living) and abiotic (non-living) factors (Jones, 2002). Of the abiotic factors, food availability and temperature are thought to be the main determinants of growth (Houde 1997; Jones 2002). For freshwater species, information regarding growth rates in relation to food availability and temperature is sparse; and for MDB fish species, there has only been one study that has examined larval growth rates with respect to food and temperature (Tonkin *et al.* 2007). Thus, gaining an improved understanding of the basic requirements for survival and growth is critical to the provision of appropriate conditions for recruitment.

This project experimentally investigated the relationship between food density and temperature on the growth and survival of the early life-stages of Murray Cod and Golden Perch. Larvae were monitored in different experimental temperature and food treatments (Table 1) from 1-2 days post hatch, until they metamorphosed to the juvenile stage.

Table 1. Experimental treatments by species. Note that food density is shown as density after feeding and average density over a 24-hour period (in parentheses).

	Murray Cod	Golden Perch
Temperature	16°C, 19°C, 22°C, 25°C	16°C, 20°C, 24°C, 28°C, 32°C
Food (density/L)	10 (6), 100 (60), 1000 (598), 2500 (1495), 5000 (2990)	10 (6), 100 (60), 1000 (598), 2500 (1495), 5000 (2990)

2.4.2 Research Outcomes Summary

Murray Cod

Our experiments demonstrated a strong interaction between temperature and food density in relation to both survival and growth of Murray Cod. Whilst temperature affected time to first feed, all larvae survived until exogenous (external) feeding commenced (9-15 days). Beyond this time, survival rates were high at colder temperatures (88.5% at 16°C) and declined at higher temperatures (34.2% at 19°C and 29% at 22°C and 25°C). At 16°C, survival was not influenced by the density of available food. In contrast, survival in the higher water temperatures was significantly influenced by food density. As temperatures increased, the amount of food required to sustain survival also increased. In direct contrast to the survival results, growth was extremely low at 16°C and much higher at 22-25°C once food densities reached 1000 zooplankton per litre.

Golden Perch

Survival rates for Golden Perch were much lower than for Murray Cod. Consequently, there were too few remaining larvae to make a robust assessment of differences in growth. The survival rates of Golden Perch were significantly influenced by both temperature and food densities. Time to first feeding varied depending on temperature. Mortalities were high prior to the commencement of external feeding (50-80%) and were lowest at 16°C. Following the onset of first feeding the highest mortalities occurred at 16°C. Overall, survival was higher at intermediate temperatures, peaking at temperatures of 24-28°C. On average, high survival was associated with higher food densities. Examination of the interactions between

food density and temperature showed that highest survival occurred at 24°C with food densities of 2500 zooplankton/L and 28°C with food densities of 5000 zooplankton per litre.

2.4.3 Water Management Application

The results from this project have demonstrated the importance of the coincidence of appropriate food densities with appropriate water temperatures for growth and survival of young Golden Perch and Murray Cod. Murray Cod are more tolerant of cooler temperatures than Golden Perch, however growth is significantly reduced at temperatures below 19°C. Given that growth is strongly linked to survival, this has significant implications for recruitment in areas that are subject to cold water pollution. If managing for recruitment outcomes, consideration should be given to the timing of flows to provide larvae with appropriate temperatures for survival and growth.

Relatively high densities of zooplankton are required to maximise survival and growth. This is particularly true for Murray Cod if temperatures exceed 19°C. For Murray Cod, there appears to be a threshold at approximately 500-1000 zooplankton per litre after which the effect of higher temperatures on survival is reduced. Additionally, both the growth of Murray Cod and the survival of Golden Perch increase with increased density of food. Zooplankton densities are naturally highly variable, and these results suggest the need to make watering decisions based on knowledge of when and where high densities of zooplankton occur and what watering strategies promote zooplankton growth.

2.5 Understanding the feeding requirements of larval fish in the northern Murray–Darling Basin

Stephen Balcombe, Ryan Woods, Kate Hodges, Priya Muhid

2.5.1 Research Question

Knowledge of the early life-history of most fish species in the northern Murray Darling Basin (NMDB) is limited. With shared species between the southern and northern MDB management decisions are often made based on knowledge attained from southern regions. This project aimed to understand the feeding requirements of early life stage fish inhabiting the dryland systems of the NMDB, where rivers are largely comprised of series of isolated waterholes joined irregularly from unpredictable rain events. This contrasts with southern MDB rivers, some of which have perennial flow. The aims of this project were: 1. To link patterns in larval abundance to antecedent (previous) hydrology and local factors across one sampling season; 2. To describe larval diets in relation to antecedent hydrology across one sampling season. The outcomes from this work provide new knowledge into early life-history stages of NMDB fish.

2.5.2 Research Outcomes Summary

This project was undertaken during severe drought (sampling occurring 2016-2018). Sampling occurred in an already flow-stressed system (from major water resource development). Sampling for fish larvae under these conditions revealed not only how limited breeding events are (in terms of reproductive output), but also how waterhole type influences breeding.

Although spawning and recruitment was limited in these isolated and disconnected waterholes, all likely resident fish species did breed in a limited way during drought. This “trickle-breeding strategy” may be crucial to maintaining populations during extended droughts. It was noteworthy that continued larval presence was found in the larger, more permanent waterholes. These waterholes have greater habitat diversity than smaller more intermittent ones, and likely contain more breeding adults. These larger waterholes confer resistance to the residing fish during harsh conditions and the resilience to support the fish populations, so they can capitalise when the next flow event occurs.

The notable outcome from the larval abundance sampling, was the incredibly low abundance and diversity of species compared to previous studies in other similar dryland rivers that go through the same boom and bust events. Previous sampling in rivers such as the Warrego and Moonie Rivers consistently produce far greater abundances (i.e. more than one order of magnitude) and diversity of larvae in both non-flow and post-flow events. This lack of abundance and diversity is likely in part due to low standing stocks of adult fish species; the large-bodied, iconic species such as freshwater catfish (*Tandanus tandanus*), Golden Perch and Murray Cod. Hence, it is likely recruitment will be limited by lack of spawning density due to low breeding adult abundances. In a regulated system such as the Lower Balonne low standing stocks will be compounded by water diversions interacting with multiple barriers impacting ability for fish to recolonise when reconnecting flows occur.

2.5.3 Water Management Application

The maintenance of key waterholes in during dry and especially extended dry periods to maintain adult fish stock is a key recommendation of this project. Distances between waterholes with large tracts of dry-channel in-between means that maintaining water levels in these dryland river systems with environmental water may not be achievable. More practical solutions such as restricting extraction (e.g. pumping rules) from key waterholes may be a better water management strategy. This necessitates clear guidelines be developed to prioritise which waterholes should be maintained and protected. Such prioritisation would have to account for more than just permanence. For example, habitat diversity of in-channel and riparian habitat would likely be more important for maintaining a resilient population of fish. Further, in agricultural

systems such as the Lower Balonne, other options may also include some habitat restoration to help future proof these waterholes against droughts and climate-change.

Other priorities should also include protecting first flush events and improving connectivity throughout the channel network to allow for recolonization of biota after extended dry events. This could entail the removal of redundant barriers and/or use of environmental flows (such as piggy-backing on natural flow events) to provide sufficient duration for fish passage upstream and downstream.

2.6 Comparison of the thermal and nutritional regimes among main channel and floodplain habitat patches

Amina Price, Paul Humphries, Rochelle Petrie

2.6.1 Research Question

Floodplains are comprised of a range of different habitat types, ranging from intermittently or permanently flowing creeks and anabranches to permanent and ephemeral wetlands. Variability in zooplankton production among floodplain habitats may occur in relation to both the degree of retention and the level of permanence of the habitat (Górski *et al.* 2013; Hein *et al.* 2004). Previous research in the mid Murray region found that zooplankton densities were higher in permanent floodplain wetlands compared to the adjacent river channel, and that water temperatures were higher on average, but far more variable than in the main channel (Beesley *et al.* 2011). However, this study only sampled relatively small permanent oxbow wetlands different floodplain habitats types such as anabranches were not included.

This project compared water temperature and zooplankton density (prey for larval fish) across six semi-permanent wetlands, anabranch and main channel sites with the aim of understanding the relative conditions for early-life stages of fish in different floodplain habitats. Sampling was undertaken on the lower Ovens River floodplain in NE Victoria on three sampling occasions encompassing the peak recruitment period for native fish.

2.6.2 Research Outcomes Summary

Temperature was measured at half-hourly intervals using loggers which were set in the top and the bottom 5cm of the water column between December 2016 and February 2017. When measured this way, the only instance in which floodplain habitats were significantly warmer than river channels, was maximum daily temperatures in the pelagic zone. Minimum daily temperatures in pelagic zone and all temperatures in the benthic zone were warmer in the river channel.

Zooplankton (rotifers and microcrustaceans) densities were significantly higher in the floodplain habitats (both wetlands and anabranches) than in the river channel (Figure), and this was consistent across all sampling occasions. Both rotifers and microcrustaceans occurred in significantly higher densities in the benthic zone than in the pelagic zone.

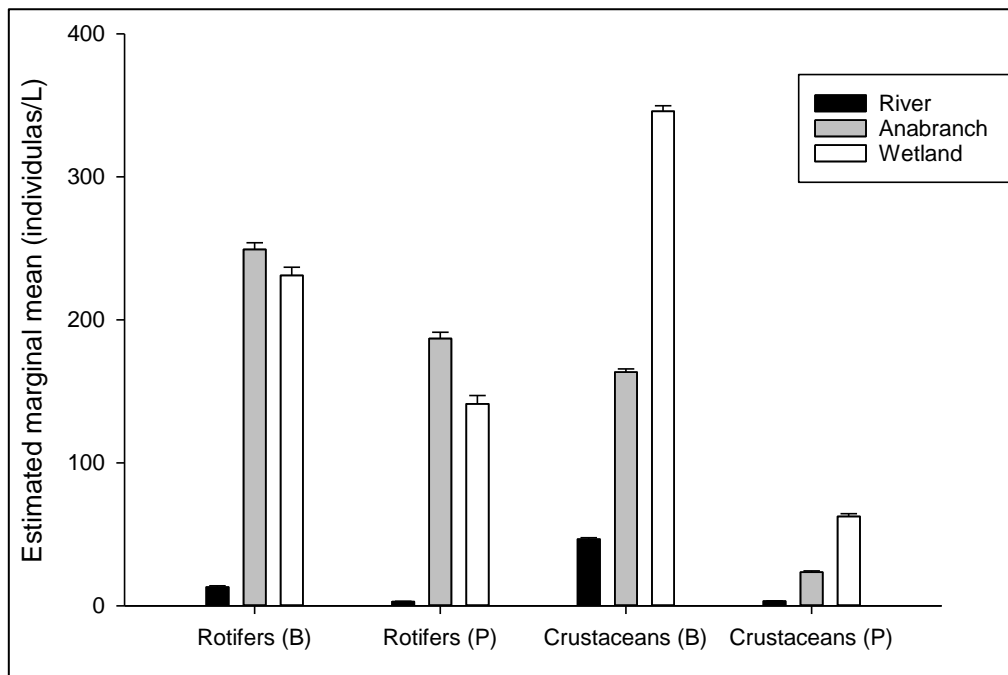


Figure 7. Average densities (+1 standard error) of benthic rotifers (B), pelagic rotifers (P), benthic microcrustaceans (B) and pelagic microcrustaceans (P) in the main channel (river), anabranches and wetlands.

2.6.3 Water Management Application

Water Management Application

Floodplains have often been highlighted as ‘warmer’ and more stable water temperature environments than the main river channel (e.g. (Jeffres *et al.* 2008; Sommer *et al.* 2001; Tonolla *et al.* 2010)); and therefore, perhaps more suitable for larval fish growth. Unexpectedly, our study found that floodplain habitats are not characterised by warmer water temperatures than river habitats. We think the discrepancy is most likely due to how water temperatures have previously been measured, as isolated pelagic only spot measurements, compared to the longer and more depth integrated approach taken here.

The results from this project provide further support that larval food (zooplankton) densities are higher in floodplain habitats than in the river channel. Furthermore, this work has highlighted the potential for anabranches to provide alternative habitats with high food densities. Anabranches often have the capacity to connect to the main channel at lower flows than floodplain wetlands so may be more frequently accessible for fish. Thus, considering anabranches may allow managers to enable access to habitats with high food densities at lower flows than those required to inundate wetlands.

2.7 The relationship between discharge, reach hydraulic complexity, zooplankton production and retention in a lowland river system

Paul Humphries, Luke McPhan, Amina Price, Geoff Vietz, Rochelle Petrie

2.7.1 Research Question

This project tests key elements of Foundational Activity 2: Riverscape Recruitment Synthesis model. The activity comprised two components: 1) assessing the relationship between reach physical complexity and flow retentiveness, and 2) assessing the relationship between flow retentiveness and the density and composition of zooplankton, in a lowland river.

The specific questions were: 1) what is the relationship between reach physical complexity and flow retentiveness and how does this change with discharge; and 2) what is the relationship between the density and composition of epibenthic and pelagic zooplankton in a lowland river and reach retentiveness, and how does this relationship change with discharge?

Based on the Riverscape Recruitment Synthesis model, we hypothesized that:

1. At moderate discharge, there will be a positive relationship between physical complexity of a river reach and measures of its flow retentiveness;
2. At lower discharges, this relationship will break down, because all reaches will converge towards higher, and so overall more homogenous retentiveness;
3. Density of benthic and pelagic zooplankton will be positively related to a gradient of reach retentiveness, but this relationship will be discharge-dependent.

This research was carried out in the Ovens River, a free-flowing lowland river and tributary of the Murray River, between December 2017 and March 2018.

2.7.2 Research Outcomes Summary

Reach physical complexity and flow retentiveness

- As hypothesized, at moderate discharge (1300 ML/day) in the Ovens River, there was a significant positive relationship between an index of reach complexity and reach flow retentiveness.
- Also as hypothesized, this relationship broke down as discharge declined, and was not present at reduced discharges of 1000 ML/day or 600 ML/day.
- Reaches did become more homogenous in their retentiveness, but not because all reaches increased in retentiveness as discharge declined, as expected. Instead, those reaches with initially high retentiveness became less retentive as discharge declined, whereas those reaches with initially low retentiveness, became more retentive as discharge declined. Thus, our hypotheses were supported, but not in way that we expected.

Zooplankton, reach retentiveness and discharge

The zooplankton fauna in the Ovens River from December 2017-March 2018 was dominated by rotifers (typically >50%), with micro-crustaceans making up the remainder. Cladocerans and copepods comprised the majority of the latter.

The density of pelagic zooplankton per reach (5-175 ind per L) was consistently much lower than the density of epibenthic zooplankton per reach (200-2000 ind per L), for each of the four months when sampling was carried out.

Densities of zooplankton were not uniform throughout river reaches: mean densities of pelagic and epibenthic zooplankton varied among reaches by a factor of more than 10.

The hypothesis that mean density of zooplankton would be positively related to a gradient of retentiveness was partially supported by analysis of the combined zooplankton data. An hypothesis test (using general linear models) indicated that there was a significant effect of including 'retentiveness' as a factor in the model.

However, when all other factors were included in the model, temperature and conductivity were found to be the most influential factors affecting density and composition of the zooplankton fauna in the Ovens River.

This means that although reach retentiveness does significantly influence density and composition of zooplankton, other factors moderate this relationship substantially.

The main zooplankton groups contributing to the effect of retentiveness, temperature and conductivity were micro-crustaceans (cladocerans, copepods and ostracods).

2.7.3 Water Management Application

This study supports the predictions from the RRSM that as discharge declines, overall reach retentiveness increases and becomes more homogenous. However, reach complexity and flow-retention relationships are reach-specific. For example, as discharge declines, some reaches become more confined to a narrower channel and so retentiveness decreases. For other reaches, as discharge declines, more slackwater areas are created and so retentiveness increases. Whilst retentiveness can be manipulated by flow and non-flow manipulations (e.g. increasing density of snags, channel form alteration), managers need to consider each reach separately and understand the existing geomorphology and physical complexity of the reach before any manipulations should occur.

Furthermore, retentiveness alone is not the biggest driver of zooplankton density or composition. Temperature and conductivity are instead the major drivers. Highlighting the likely impact of thermal (cold-water) pollution from bottom-release dams on reduced zooplankton production in lowland rivers.

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

Lorena Bettinelli Nogueira (PhD student), Susan Lawler, Paul Humphries, Amina Price

This project is part of Lorena's PhD at La Trobe University which will finish in 2020. Therefore only preliminary results are included here.

2.8.1 Research Question

This study aimed to improve the understanding of larval dispersal of three iconic Australian freshwater fish, Golden Perch (*Macquaria ambigua*), Murray Cod (*Maccullochella peelii*), and Trout Cod (*Maccullochella macquariensis*), by investigating swimming behaviour (swimming activity, orientation towards the flow direction and direction of movement) under a range of simulated flow conditions. A racetrack flume, capable of producing a gradient of flow velocities, was used to simulate different flow conditions that are present in the Murray River, hereafter classified as i) fast; ii) moderate; iii) slow; iv) regulated scenarios (weir pool). Four consecutive developmental stages were released in the flume at each flow scenario and their movement and behaviour tracked for five minutes using a video camera.

2.8.2 Research Outcomes Summary

In general, Golden Perch chose to swim near the bottom of the flume, while the two Cod species swam closer to the surface or in the middle of the water column.

Swimming behaviour showed that during downstream movements, most individuals were oriented upstream, with active swimming behaviour in all three 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, where fish are moving against the current, was observed for all three species, with the older life stages moving significant distances. Active-downstream movements, where the fish were oriented downstream and swimming faster than the current, were also identified. This means is that all fish larvae tested so far are clearly non-passive, that is they orient themselves and move between fast and slow currents thereby influencing their path of movement.

2.8.3 Water Management Application

When planning environmental releases, it is important to ensure that the water is moving when larval fish need to disperse. Future work in this area will allow us to identify threshold velocities that trigger dispersal of native larval fish.

We found that in the regulated scenario (which simulates a weir pool), Golden Perch larvae swam to the bottom and found a place to sit still. This has implications for river regulation, suggesting that a series of reservoirs will present significant challenges to Golden Perch larval dispersal. Dam operators may be able to manage flows in a way that enhances dispersal for this species (e.g. by moderating flow when fish are likely to be moving to ensure fish low in the water column can move past structures).

2.9 Investigating the relationship between flow, structural habitat, hydrodynamics and patterns of larval settlement and retention

Paul Humphries, Stacey Kopf, Jarrod Chapman, Amina Price, Nicole McCasker, Keller Kopf, Matthew McLellan, Dale Campbell

2.9.1 Research Question

This activity aims to quantify the relationship between flow and reach-scale retentiveness (ability to retain objects within the water due to slack-water, structure or other barrier) of fish larvae of species with different swimming abilities. This was done by using field-based experimental releases of Murray Cod and Golden Perch larvae and comparing their movement to passive particles (i.e. particles that cannot influence their response to flow). The project investigated the relative retention of the larvae of a strong-swimming species (Murray Cod) and a poor-swimming species (Golden Perch) in reaches of differing retentiveness, and how this was affected by discharge.

The questions were: 1) how do proportions of recaptured versus released larvae of Murray Cod and Golden Perch compare to the proportions of recaptured versus released passive particles in reaches of differing retentiveness; and 2) how does discharge influence the proportions of recaptured vs. released larvae of Murray Cod and Golden Perch compare to the proportions of recaptured vs. released passive particles in reaches of differing retentiveness

We hypothesize that:

1. That the proportion of Murray Cod larvae relative to passive particles drifting through reaches will always be higher than that of Golden Perch larvae;
2. That for Murray Cod, the proportion of larvae relative to passive particles drifting through reaches will decrease as reach retentiveness decreases;
3. That for Golden Perch, the proportion of larvae relative to passive particles drifting through reaches of differing retentiveness will be relative constant, and close to 1;
4. That for Murray Cod, the proportion of larvae relative to passive particles drifting through reaches will be lower at moderate than for relatively high discharges;
5. That for Golden Perch, the proportion of larvae relative to passive particles drifting through reaches will be similar at low and relatively high discharges, and close to 1.

Larval release experiments were carried out in the Ovens River in November 2018 (Murray Cod), December 2018 (Murray Cod and Golden Perch) and February 2019 (Golden Perch), releasing 140,000 marked larvae, sourced from NSW hatcheries.

2.9.2 Research Outcomes Summary

As hypothesised, when released simultaneously, a greater proportion of the larvae of Murray Cod than Golden Perch drifted through all reaches. This meant that a greater proportion of Golden Perch than Murray Cod were retained in all reaches.

In most cases, more larvae of both species were retained in reaches, regardless of reach retentiveness, than passive particles. This strongly suggests that Murray Cod and Golden Perch larvae – fish with very different swimming abilities – are not passive in their movements through lowland river reaches but have some capacity to determine their location.

At moderate discharge (1200 ML/day), the relative proportions of recaptured Murray Cod larvae to passive particles varied negligibly with reach retentiveness. Fewer Murray Cod larvae than passive particles were recaptured in each reach, i.e. more Murray Cod than passive particles were retained in all reaches.

At higher discharges (2000-4000 ML/day), the relative proportions of Murray Cod larvae to passive particles changed with reach retentiveness. In reaches with high retentiveness, Murray Cod larvae were retained at greater rates than passive particles, whereas when reach retentiveness decreased, Murray Cod larvae were

retained at similar rates to passive particles. However, for the two reaches with the lowest retentiveness, there were contradictory results: in one reach, almost 8 x more Murray Cod larvae than passive particles drifted through the reach, whereas in the other reach, 22 x more passive particles than Murray Cod drifted through the reach.

At low (350 ML/day) and moderate (2000-4000 ML/day) discharges, the relative proportions of Golden Perch larvae to passive particles decreased with increasing reach retentiveness. This was most pronounced at the lower discharge. Golden Perch larvae were being retained more than passive particles at relatively low reach retentiveness and were being retained less (or had greater dispersal through the reach) as reach retentiveness increased.

These results suggest that at moderate and relatively high discharges, Murray Cod larvae can determine whether they stay or move through a reach.

At low and relatively high discharges, Golden Perch larvae are behaving more like passive particles than do Murray Cod larvae, but still have a degree of capacity to determine whether they stay or move through a reach.

2.9.3 Water Management Application

These results unequivocally support the notion that Murray Cod and Golden Perch do not drift wholly passively, i.e. at least in some flow conditions they may choose which habitats they settle into or disperse through. Management actions such as removing fish passage barriers or flow manipulations should consider the dispersal abilities of young fish.

Highly flow-retentive reaches are likely to be the most retentive for drifting fish larvae; with large numbers of larvae potentially able to be retained in relatively short sections of lowland rivers. Overall, in-channel physical complexity provides the basis for food production and retention for young fish. If retention is uniformly high throughout a reach, then a larva has little choice but to remain. If retention is uniformly low, it seems that larvae have some capacity to remain or drift. But in these situations, such reaches may not be very conducive for feeding, growth, survival and recruitment – although this is less certain and needs more investigation.

If managers want to maximise the potential recruits in a section of river, then increasing physical complexity of that reach, or altering flows to increase retentiveness, will increase the likelihood of fish larvae being retained. Maintaining a diversity of hydraulic, structural and geomorphic conditions in river reaches will allow fish larvae to be retained or move through, if they choose.

2.10 Basin-scale population dynamics of Golden Perch and Murray Cod: relating flow to provenance, movement and recruitment in the Murray–Darling Basin

Brenton Zampatti, Ben Fanson, Arron Strawbridge, Zeb Tonkin, Jason Thiem, Gavin Butler, Stephen Balcombe, Wayne Koster, Alison King, David Crook, Ryan Woods, Steven Brooks, Jarod Lyon, Lee Baumgartner, Katherine Doyle

2.10.1 Research Question

Restoration of riverine connectivity and ecologically relevant aspects of natural flow regimes are fundamental to rehabilitating riverine fish populations. To be effective, however, this requires an understanding of relationships between flow and the key life history processes (e.g. spawning, recruitment and movement) that influence population dynamics. The MDB is a large and complex river system, characterised by a climatically and hydrologically varied riverscape. In such systems, specific regions may act as ‘sources’ and ‘sinks’ for various life stages, and connectivity between regions may be an important determinant of population variability. Consequently, understanding the provenance of early life stages (i.e. location of birth and early growth), and subsequent recruitment and dispersal, is essential for management and conservation.

Golden Perch and Murray Cod are the largest and longest-lived native freshwater fishes in the MDB. Populations of both species have declined in abundance and range, due to altered flow regimes, fragmentation and overharvesting, amongst other factors. To arrest decline and rehabilitate populations, the reproduction, recruitment and movement of both species form primary objectives for environmental water allocations throughout the Basin. Nevertheless, despite many years of water delivery and monitoring, it is still unknown at what spatial scale the processes that govern the dynamics of populations operate, or if they are associated with flow.

In this project we aimed to: 1) investigate the spatial scales at which Golden Perch and Murray Cod populations operate in the MDB, and 2) clarify spatio-temporal relationships between flow and key population processes for both species. To achieve this, we used the structure and chemistry of fish otoliths (ear bones) to develop an environmental timetable for individual fish that allowed us to identify which habitats a fish had moved through during its life. Fish otoliths are much like the rings in the trunk of a tree; they grow annually, and each ring can tell us about the conditions a fish was in at a specific time. The material that makes up the otoliths comes from the environment in which the fish is living. Scientists can tell where a fish was by examining the Strontium isotope in the otolith and matching its signature to a specific place.

Our specific objectives were to:

1. Investigate spatial and temporal variability in the water $^{87}\text{Sr}/^{86}\text{Sr}$ isoscape (variation in Strontium isotopes has a signature depending on where those isotopes are collected) of the MDB. This is fundamental to developing a template to clarify the spatial origin of fish.
2. Determine regional age structures and use otolith chemistry to determine the provenance (birth year and place) for Golden Perch; and movement history of Golden Perch and Murray Cod from each region and relate these to environmental conditions (particularly flow and water temperature) at appropriate scales.
3. Integrate these data to develop a river-scale understanding of life-history, movement and population dynamics, and response to flow.
4. Use this understanding to inform the spatial scale of environmental water management.

2.10.2 Research Outcomes Summary

There was a clear delineation of $^{87}\text{Sr}/^{86}\text{Sr}$ in water samples ($n = 726$) between the Murray and Darling River catchments (collected between 2011-2018), and to a lesser extent within these river catchments (Figure 7). This enabled us to have confidence in assigning provenance based on isotope composition of the otoliths.

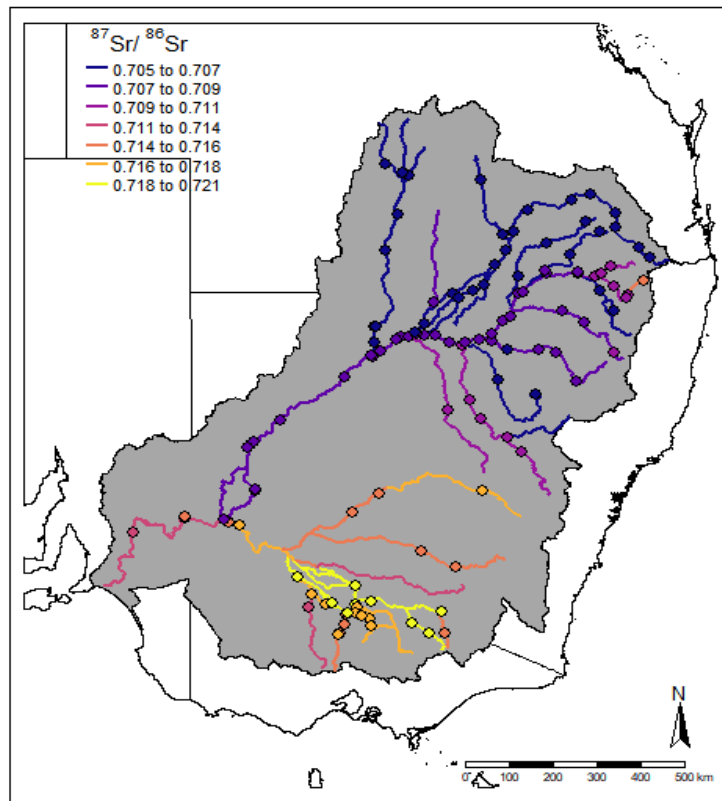


Figure 7. Variation in water $^{87}\text{Sr}/^{86}\text{Sr}$ (coloured lines) across the major connected rivers of the MDB. Colour-filled circles represent the locations of measured water $^{87}\text{Sr}/^{86}\text{Sr}$ samples.

Golden Perch age demographics

Age population structure (demographics, $n = 856$) of Golden Perch collected in 2018 from 14 rivers across the MDB indicated substantial variability in the population demographics (including recruitment) (Figure 8). For example, few young fish occurred in the Lower Murray, Goulburn and Upper Murray, whereas high numbers of young fish occurred in the Lower Darling and Condamine/Balonne catchments relative to other age classes.

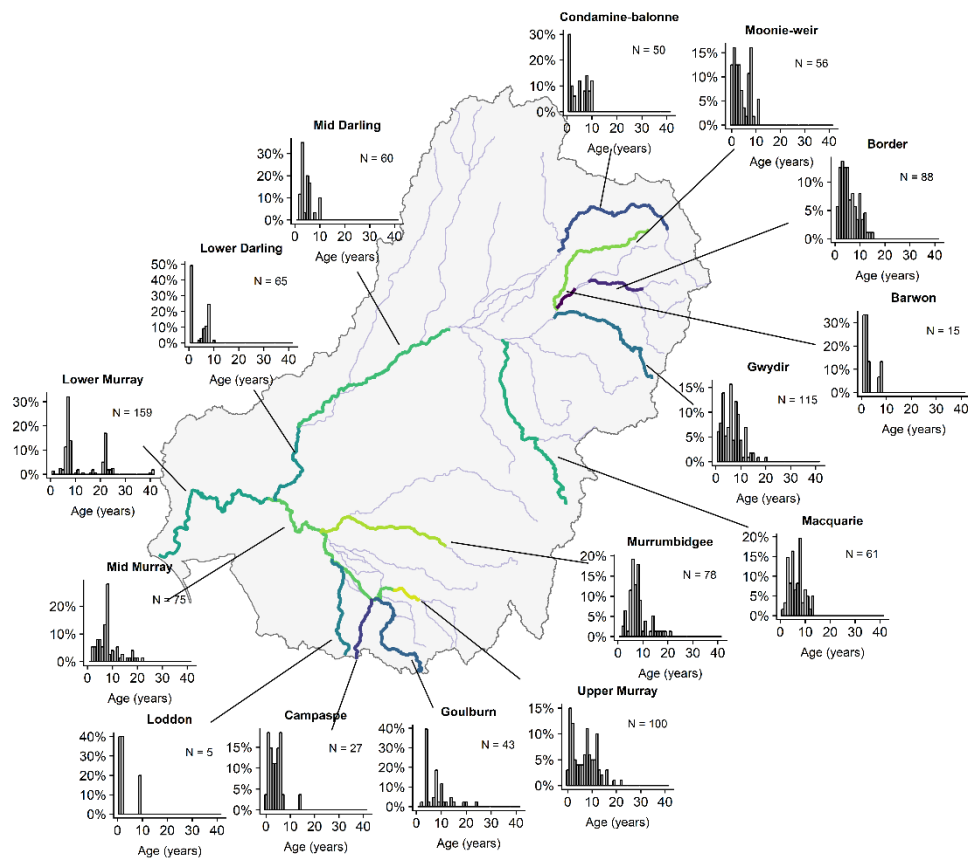


Figure 8. Age structures of golden perch ($n = 856$) collected from 14 regions across the Murray-Darling Basin in 2018.

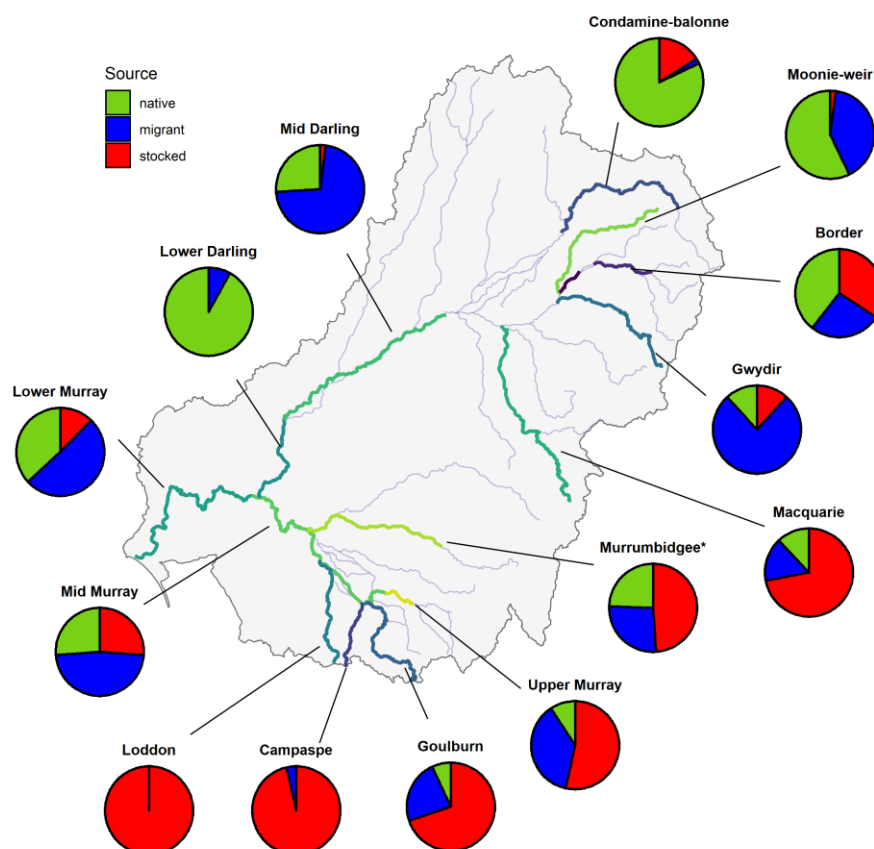


Figure 9. Map depicting the natal origin of golden perch comprising the sampled populations from 14 regions across the MDB in 2018. Green = fish native to the region, blue = immigrants and red = stocked.

Natal origin and movement of Golden Perch and Murray Cod

For Golden Perch, across the MDB there was substantial variation in the influence of *in situ* recruitment (native), movement (migrant) and stocking on regional population structure. For Murray Cod, *in situ* recruitment and movement were the principal determinants of population structure in rivers of the northern and southern Basin (Figure 9).

Determination of the natal origin of individual Golden Perch enabled the development of spatially reconciled age-structure based on recruitment source. This highlights the dominance of hatchery bred fish in the system, the lack of recruitment over many years in some river catchments (e.g. Gwydir, Macquarie, Loddon, Campaspe and Goulburn), and the strength of other regions such as the Lower Darling as a natural recruitment source. These data will form the basis of analysis associating recruitment (year-class strength) with environmental variables (including flow) at the place and time of birth.

Flow-recruitment analysis

We used the spatially reconciled age structure for Golden Perch (stocked fish removed and migrants assigned to their birth river) to assess flow-related recruitment dynamics across the MDB. Overall, we found a significant positive effect of *spawning flow pulse* (change in flows during the core spawning period) on year-class strength (YCS), but only in the southern Basin. Specifically, for the southern MDB, the model predicted that a doubling of the flow pulse during the prescribed spawning season (October–March) resulted in a 64% increase in year class strength (95%CI 14% to 136%).

2.10.3 Water Management Application

Golden Perch

- Golden Perch production and demographics are not homogenous across rivers of the MDB, but no region appears a dominant recruitment source.
- In the northern Basin, the Moonie, Condamine-Balonne and Border rivers have had consistent recruitment over the past decade. In the southern Basin, the lower Darling, lower, mid and upper Murray and Murrumbidgee rivers are apparent recruitment sources, but recruitment may be episodic, particularly in the lower and upper River Murray, and lower Darling River. These rivers may contribute fish to other rivers via emigration.
- Regions of low recruitment include the Gwydir, Macquarie, Goulburn, Campaspe and Loddon rivers. In these regions, population persistence is largely influenced by stocking (i.e. artificial rearing in hatchery conditions and release of juveniles). Additional scrutiny is required of regions where in situ recruitment appears low and where stocking may dominate (e.g. Macquarie, Loddon, Campaspe and Goulburn rivers). In these regions, the mechanisms promoting poor recruitment and/or immigration warrant further investigation. Furthermore, whilst in situ recruitment may be poor in some rivers, these may still act as a source of recruits to adjacent rivers (e.g. the Goulburn River which is a source of fish to the mid and upper River Murray).
- Immigration (movement from other regions) is a strong driver of Golden Perch population dynamics in some regions.
- Along with inter-catchment movement of juveniles and adults, low levels of continuous recruitment across large regions of the MDB likely promote population persistence across a riverscape characterised by environmental heterogeneity.
- Our preliminary flow-recruitment analysis indicated that, in the southern Basin, the greater the magnitude of a flow pulse during the spawning season (October–March), the greater recruitment is likely to be. In the northern basin, however, a similar relationship was not evident.
- Regions with age-structure diversity are likely to be more resilient to environmental perturbations and may improve future spawning and recruitment.
- Golden Perch population dynamics across the MDB are influenced by the interaction of populations in rivers that may be separated by 100s–1000s km. Environmental water management that aims to promote Golden Perch population growth needs to consider the scale at which this metapopulation operates, and the need for hydrological and physical connectivity for all life stages. This is also pertinent for monitoring of environmental flow outcomes.

Murray Cod

- Murray Cod population structure was influenced by in situ recruitment and emigration/immigration. Movement, however, was generally restricted to an adjacent river catchment/reach or anabranch.
- Stocking had no discernible influence on the population structure of Murray Cod.
- Murray Cod population contingents appear to function of scales of 10s–100s km, hence environmental water management to support Murray Cod population growth could potentially be undertaken at the individual catchment scale. Concurrently, however, the interaction of contingents in adjacent catchments and need for connectivity also warrants consideration.

For both Golden Perch and Murray Cod, connectivity between tributary and mainstem habitats can be a substantial driver of population structure. Where present, maintaining the unique hydrologic and habitat characteristics of tributaries or mainstem rivers is paramount, as is facilitating connectivity between tributary and mainstem population contingents to ensure metapopulation integrity.

3 Theme Synthesis

This series of interlinked research studies has shed new light on the mechanisms and drivers of fish recruitment in the MDB. The research deliberately undertook a scaled approach to explore the mechanisms behind some of the varied responses of fish recruitment to flow observed in previous studies and monitoring programs. Understanding the causes of this variability should enable better predictions about the likely responses to future managed and unmanaged flows, environmental watering and other non-flow related management interventions.

Our research shows that population processes particularly governing recruitment, operate at different spatial scales for different species. Nevertheless, regardless of the scale at which populations operate, recruitment is dependent on patch to segment scale processes that determine the food and temperature regimes and the ability of larvae to either disperse or be retained in suitable rearing areas. Our research also shows that under certain circumstances, such as during drought conditions, additional factors are critical in determining recruitment success.

The research approach and outcomes of the EWKR Fish Theme has developed throughout the program, and we have not only addressed the original research questions but have also evolved outcomes not originally intended. We believe the research has a few key themes that are critical when considering the recruitment of fishes in the MDB, and these are discussed below.

3.1 Life history strategy influences spatial scale of population dynamics

Winemiller and Rose (1992) developed a triangular life history model that proposes three conceptual endpoints or groups of fishes: opportunistic, periodic and equilibrium (Table 2). The model proposes that factors such as longevity, age at maturity, fecundity and offspring size interact with environmental conditions to ensure the best chance for the survival of young. This model has been previously applied to MDB fishes (e.g. Humphries et al. 1999; King et al. 2013). Given the importance of life-history strategy for fishes, the Riverine Recruitment Synthesis Model (section 2.3) developed in this program, predicts recruitment responses to flows will vary among species with different life-history strategies. Variability in recruitment will arise because of differences in food, habitat and the requirement and capacity for movement or retention among life-history strategies.

Table 2. Key species traits and examples of MDB fish species for opportunistic, periodic and equilibrium groups.

	Opportunistic	Equilibrium	Periodic
Key traits	Small-bodied; low fecundity; fast-hatching; small yolk-sac; small larvae and small size at first feeding; poor larval swimming ability	Moderate to large-bodied; low fecundity; slow hatching; large yolk-sac; large larvae and large size at first feeding; good larval swimming ability	Moderate to large-bodied; very high fecundity; fast-hatching; small yolk-sac; small larvae and small size at first feeding; very poor larval swimming ability
Population scale	Site to reach	Segment to catchment	Catchment to basin
Examples of MDB species	Australian smelt (<i>Retropinna semoni</i>) carp gudgeons (<i>Hypseleotris spp.</i>) bony herring (<i>Nematolosa erebi</i>) Murray river rainbowfish (<i>Melanotaenia fluviatilis</i>)	Murray Cod (<i>Maccullochella peelii</i>) trout Cod (<i>Maccullochella macquariensis</i>) freshwater catfish (<i>Tandanus tandanus</i>)	Golden Perch (<i>Macquaria ambigua</i>) Silver Perch (<i>Bidyanus bidyanus</i>)

Our research has demonstrated that the population dynamics of species from different life-history strategies are likely to operate at different spatial scales (Table 2). Whilst not investigated as part of this research, opportunistic species are able to complete their life-cycle and maintain populations at the site to reach scale. The population dynamics of Murray Cod, a representative of the group of equilibrium species, operate at scales of 10s–100s km, hence environmental water management to support Murray Cod population growth could potentially be undertaken at the individual catchment scale. In contrast, the population dynamics of Golden Perch, one of only a few periodic species that occur in the MDB, occurs at a whole of Basin scale. Environmental water management that aims to promote population growth needs to consider the scale at which metapopulations operate, and the need for hydrological and physical connectivity for all life stages.

3.2 Interaction of food and temperature at small scales governs growth and survival of young fish

At patch scales, the survival and growth of fish larvae is strongly influenced by the interaction between food density and temperature. Our results show that survival rates can vary substantially depending on food and temperature conditions. For both Golden Perch and Murray Cod, survival and growth were maximised at high food densities (above 500-1000 zooplankton/L). Optimal temperatures for larval growth and survival, however, varied between the two species and may reflect the different spawning times of the species. Of note, were the very low survival rates of Murray Cod at warm temperatures when food densities were below 500-1000 zooplankton/L. This finding has implications for the species under climate change.

At the patch scale, zooplankton densities have been shown to be affected by the retention time of water within the patch, with higher zooplankton densities found in still- and slow-flowing areas such as slackwaters (King 2004; Humphries et al. in press; Ning et al. 2010). Whilst there is little capacity for managers to manage individual patches in the riverscape, maintaining or improving hydraulic diversity within and among reaches improves the likelihood that high retention patches will be present under a range of flow conditions. As water temperatures at the patch scale are also affected by retention time, as well as water depth and shading (Ning et al. 2010), management activities targeting hydraulic diversity and riparian zones could be influential and require further research confirmation.

3.3 Food and temperature regimes vary at the site scale based on connectivity to the main channel

Our research shows that zooplankton occur in much higher densities in the benthic zone (see also King 2004), and that when comparing zooplankton densities among floodplain habitat types, densities are significantly higher in wetlands (103-1494 zooplankton/L) and anabranches (57-721 zooplankton/L) than in the main river channel (5-55 zooplankton/L). Based on our experimental manipulations for Murray Cod and Golden Perch survival and growth, these results indicate that suitable growth and survival conditions for these larvae should occur with access to floodplain wetlands and anabranches. Furthermore, our results highlight that anabranches may represent an important habitat for the provision of high food densities for young fish, that have been previously largely overlooked for water manipulation for fish rearing.

Our results have also demonstrated that for most of the summer period, water temperatures were warmer and more stable in the main river channel, than compared with wetlands and anabranches. This result counters the widely-held belief that floodplain environments are warmer than main channel environments (King et al. 2003), and has probably stemmed from sampling inaccuracies and inefficiencies. This result suggests that main channel environments may provide improved thermal conditions for recruitment of some species.

3.4 Under dry or drought conditions in the Northern MDB, recruitment is more strongly related to the distribution of adults, and less related to food and temperature

Our research in the Northern Basin, which took place during drought conditions, demonstrated that despite high food densities and warm temperatures occurring in disconnected waterholes, the diversity and abundances of larval fish were low. Spawning and/or recruitment was thought to be limited by both a lack of adults within the waterholes and limited flows. Whilst, larvae were continually present in the permanent waterholes that were larger and deeper, peaks in larval abundances occurred with small flow rises. This is likely due to high habitat diversity in these larger waterholes, compared to smaller, less complex waterholes. Large waterholes are likely to confer resilience to resident fish during harsh conditions.

This research also highlights the need to identify, maintain and protect critical refuges during dry conditions. Once wetter conditions return, consideration must be given to the provision of flows, in conjunction with connectivity, to ensure that fish are able to move between waterholes and throughout the system.

3.5 Heterogeneity of hydraulic conditions at reach scales enable growth and survival of young fish

The early-life stages of many fish species occur within the main channel, either because they require flowing water or because they do not have access to floodplain habitats. Despite the relatively low densities of food (zooplankton) in the main channel, larval fish survival and growth may still occur if high retention patches (e.g. slackwaters, snags, macrophytes) are present within the main channel (King 2004; Schiemer et al. 2001).

Our research has shown that retention varies at the reach-scale and that this influences zooplankton densities and community composition. Reach-scale differences in retention are driven by an interaction between physical complexity and discharge. This relationship is particularly important at moderate discharges when flows are high enough to create large amounts of flowing water, but not high enough to result in the connection of floodplain habitats.

3.6 Heterogeneity of hydraulic conditions at reach scales enables dispersal and retention of young fish

Our research demonstrates that at reach-scales, flow retention and discharge influences the dispersal and/or retention of fish larvae through a reach. These relationships varied between Golden Perch larvae (relatively poor swimming ability) and Murray Cod larvae (relatively good swimming ability). For example, a greater proportion of Golden Perch larvae were retained within reaches, indicating greater capacity for Murray Cod to actively drift. Compared to passive particles, however, fish larvae of both species were generally retained in all reaches. This suggests that the larvae of both species are not passive in their movements through lowland river reaches but have some capacity to determine their location.

Generally, more larvae of both species were retained in hydraulically, structurally and geomorphically complex reaches. Maintaining this diversity of complexity among river reaches will allow fish larvae to be retained if conditions are suitable or move through to avoid adverse conditions or to disperse to more favourable conditions.

3.7 Diversity and connectivity at catchment to basin scales are critical for maintaining populations through recruitment and migration

Our research has demonstrated that Golden Perch and Murray Cod recruitment and population demographics are not homogenous across rivers of the MDB. Murray Cod population structure was influenced by *in situ* recruitment and emigration/immigration between adjacent river catchments, reaches or anabranches. For Golden Perch, recruitment was spatially and temporally variable among rivers.

Across the MDB there is no specific region that acts as a dominant recruitment source for either Murray Cod or Golden Perch. For Golden Perch, some regions appear to support consistent *in situ* recruitment, others are characterized by episodic recruitment, and in some regions, recruitment appears to be limited. In these regions, populations are maintained by stocking and/or immigration. Along with inter-catchment movement of juveniles and adults, asynchronous recruitment across large regions of the MDB likely promotes population persistence across a riverscape characterised by environmental heterogeneity. For both Golden Perch and Murray Cod, connectivity between tributary and mainstem habitats can be a substantial driver of population structure. Where present, maintaining the unique hydrologic and habitat characteristics of, and connectivity among, tributaries or mainstem rivers is paramount to ensuring future population integrity and viability.

4 Management relevance

We have demonstrated that both fish populations and the key drivers and processes that support populations through recruitment operate at multiple spatial scales, from the patch to the basin scale. Areas of high zooplankton production, coupled with appropriate temperatures, support high levels of recruitment, and hydraulic diversity is integral to providing appropriate conditions for food production and dispersal/retention of early-life stages. Connectivity at small to large scales is also critical in enabling access to appropriate habits and dispersal to maintain populations at catchment to basin scales.

During very dry and dry conditions, large-scale recruitment events are unlikely. Consequently, key management targets should focus on maintaining populations at the site scale. As more water becomes available, consideration of hydraulic diversity and floodplain access can occur. In the first instance, floodplain access can be facilitated via anabranches, as these connect to the main channel at lower flows than wetlands. Knowledge of structural and geomorphic complexity at the reach to segment scales will enable flows to be delivered to create a range of retention conditions which in turn, will support food production and larval dispersal and settlement. In cases where there is little physical complexity, management actions may focus on improving complexity prior to focusing on flow delivery.

During very wet years, hydraulic diversity and floodplain access are more likely to occur naturally. Large-scale connectivity may be impeded by barriers to movement.

The capacity of water managers to maximise these conditions is dependent on the availability of water. Consequently, the scale at which management can occur, as well as the management outcomes that can be achieved, will vary depending on the amount of water available (Table 3).

Table 3. Management scale, potential fish recruitment outcomes, risks, key consideration and knowledge requirements under different resource availability scenarios.

	Resource Availability Scenarios			
	Very dry	Dry	Moderate	Wet to Very Wet
BWES Management objective	Avoid irretrievable loss of or damage to, environmental assets	Ensure environmental assets maintain their basic functions and resilience	Maintain ecological health and resilience	Improve health and resilience of water dependent ecosystems
Scale	Site, reach	Site, reach, segment	Site, reach, segment, catchment	Site, reach, segment, catchment, basin
Potential fish recruitment outcomes	<p>4. Site to reach scale recruitment of opportunistic species providing that predation rates are low and that water quality conditions remain favourable.</p> <p>5. Maintenance of adult breeding stock, especially for large-bodied species.</p>	<p>6. Site to reach scale recruitment of opportunistic and equilibrium species, providing that predation rates are low and that water quality conditions remain favourable.</p>	<p>7. Potential for high levels of recruitment of opportunistic and equilibrium species at site to catchment scales.</p> <p>8. Potential for spawning and recruitment of periodic species with appropriate hydrological cues (e.g. within channel flow pulses).</p> <p>9. The level of recruitment will be mediated by flow/complexity/retention relationships; patch and reach scale hydraulic diversity; capacity to enable floodplain connections.</p>	<p>10. Potential for high levels of recruitment of opportunistic and periodic species at site to catchment scales.</p> <p>11. Potential for inter-catchment movement of periodic species and within-catchment dispersal of equilibrium species. The level of recruitment will be mediated by flows that support both spawning/recruitment and the capacity to enable lateral and longitudinal connections.</p>
Risks	<ul style="list-style-type: none"> • Cyanobacteria blooms • Hypoxia events leading to fish kills • High rates of predation and competition by alien species within sites. 	<ul style="list-style-type: none"> • Cyanobacteria blooms • High rates of predation and competition by alien species within sites. 	<ul style="list-style-type: none"> • Potential for hypoxic events, dependent of flooding history. 	<ul style="list-style-type: none"> • Potential for blackwater events, dependent of flooding history.
Key considerations	<ul style="list-style-type: none"> • Limit water extraction. • Maintenance /restoration of critical habitat attributes (e.g. riparian and instream habitat features). • Reduce predation risk from non-native species. 	<ul style="list-style-type: none"> • Limit water extraction and provision of top-up flows to key refuge areas to maintain water quality and depth. • Maintenance/restoration of critical habitat attributes (e.g. riparian and instream habitat features). 	<ul style="list-style-type: none"> • Facilitate connection to anabranches and wetlands if possible • Timing of flow peaks to maximise appropriate temperature regimes. • Sufficient duration of inundation of productive habitats (both in- and off-channel) to enable rapid growth and survival of young fish • Location of flows based on capacity for dispersal, retention and hydraulic 	<ul style="list-style-type: none"> • Prioritisation of environmental water based on knowledge of basin-wide sources of recruitment of periodic species. • Timing of flows to maximise appropriate temperature regimes. • Longitudinal connectivity to provide capacity for large-

Resource Availability Scenarios				
	Very dry	Dry	Moderate	Wet to Very Wet
	<ul style="list-style-type: none"> • Restrictions on fishing/harvesting from refuge habitats. • Monitoring of critical refuges to avoid loss of source adult populations. 	<ul style="list-style-type: none"> • Maintenance of lotic conditions in perennial rivers • Reduce predation risk from non-native species. • Restrictions on harvesting from refuge habitats. • Monitoring of critical refuges to avoid loss of source adult populations. 	<div>diversity (scale is dependent on fish life history)</div> <ul style="list-style-type: none"> • In areas with poor hydraulic diversity, actions to promote enhance physical complexity (such as weir pool lowering). • Consideration of connectivity and movement barriers. 	<div>scale movements of periodic species.</div> <ul style="list-style-type: none"> • Facilitate slow flood recession to enable fish to return to permanent habitats.
Knowledge requirements	<ul style="list-style-type: none"> • Location of critical refuge habitats at catchment scales. • Understanding of critical habitat attributes that create appropriate refugia. 	<ul style="list-style-type: none"> • Location of critical refuge habitats at catchment scales. • Understanding of critical habitat attributes that create appropriate refugia. 	<ul style="list-style-type: none"> • Potential for nutrient and energy inputs (longitudinal and lateral). • Degree and variability of physical/hydraulic complexity. • Scale of lotic habitats required for recruitment of periodic species. 	<ul style="list-style-type: none"> • Potential for nutrient and energy inputs (longitudinal and lateral). • Sources of recruitment, and variability under different flow patterns and conditions. • Movement pathways. • Importance of floodplain flows for recruitment and dispersal of small-bodied floodplain fishes

5 Knowledge Status

5.1 Knowledge status

The research undertaken for the EWKR Fish Theme has highlighted the numerous drivers and processes that support fish recruitment in the MDB, and how these interact with flow. This research highlights that many of the key drivers of fish recruitment are only indirectly related to flow and therefore, the need to consider interactions between flow and other factors in management to support recruitment.

We have highlighted the importance of temperature in driving survival and growth of early-life stages, but also, the interaction between food densities and temperature. Whilst temperature is not easily 'managed' using flows, management decisions regarding the timing of flows to maximise optimal temperatures for early-life stages are likely to impact on recruitment success. In addition, cold water pollution mitigation (e.g. multiple offtakes, artificial de-stratification) could be beneficial.

In the southern MDB, food densities for larval fish (zooplankton) were found to be significantly higher in floodplain habitats than in the river channel itself. Consequently, access to high food densities could be managed by facilitating connectivity to, and from, inundated anabranches and floodplain wetlands. Within the main channel, high retention reaches in medium discharges had higher food densities for early life-stages. Suggesting that manipulations of structural and geomorphic complexity within the main channel may provide enhanced recruitment outcomes for fish. This is a key research outcome that also requires further research but highlights a potential non-flow management strategy that could benefit fish.

Our research has provided further support demonstrating that downstream drift of fish larvae of both Murray Cod and Golden Perch is not completely passive, even for poorly swimming species. This has significant implications for how larvae interact with the physical complexity and hydraulic conditions within a reach. Overall, physical complexity provides the basis for food production, retention of this food, and for fish larvae to either choose to remain or to move through river reaches. If retention is uniformly high throughout a reach, then a larva has little choice but to remain. If retention is uniformly low, it seems that larvae have some capacity to remain or drift. But in these situations, such reaches may not be very conducive for feeding, growth, survival and recruitment. If managers want to maximise the potential recruitment in a river, then increasing physical complexity of that reach, or altering flows to increase retentiveness, will allow fish larvae to remain. Maintaining a diversity of hydraulic, structural and geomorphic conditions both within and among river reaches will allow fish larvae to be retained or move through, if they choose.

During dry or drought conditions, refuges are well known to play a critical role in maintaining fish populations. Our research in the northern MDB has suggested that even when small flow spikes do occur the abundance and species diversity of larvae is limited. Under these conditions, food and temperature was not a limiting factor – but rather flow. This highlights the need to appropriately manage these areas during these tough periods, using both flow and non-flow related measures, to ensure resilience of the adult breeding stock during drought, so that strong recruitment can occur when suitable flow conditions return.

The EWKR Fish Theme research has also demonstrated the importance of understanding the spatial scale at which population processes operate within. population processes across for two iconic species. Population dynamics of Murray Cod operate at scales of 10s–100s km's; while Golden Perch operate at larger spatial scales again, 100s-1000s km's. Populations of these species are maintained by *in situ* recruitment, which is spatially and temporally variable across the MDB, as well as immigration and emigration between rivers, catchments; and in the case of Golden Perch, regions across the MDB.

A key outcome of the EWKR fish theme research is the importance of hydraulic diversity, and hydrological and physical connectivity for promoting successful fish recruitment. These concepts need to be considered from patch – catchment scales, and across the Basin. Whilst there are intrinsically flow management implications here, non-flow restoration and management measures should also be considered alongside flow.

5.2 Knowledge gaps

While the work undertaken for the EWKR Fish Theme has greatly improved our understanding of fish recruitment, the work has also identified areas that require further research. Key knowledge gaps remaining are:

- Investigation of food-temperature-retention relationships in modified systems: while our research has demonstrated the importance of hydraulic diversity and the crucial interaction between physical complexity and flow in driving this diversity, the current work was undertaken in the lower Ovens River, which is characterized by high physical complexity. To better understand these relationships, examining the interactions in more altered systems would be beneficial. Additional research is also required to determine whether these relationships vary across a range of river types (e.g. lowland, upland, ephemeral and dryland rivers).
- Exploring the capacity to remotely measure instream physical complexity to enable managers to make relatively rapid assessments.
- Test the predicted relationship that fish recruitment success is improved with increased retention and physical complexity within main channel flows.
- Identify and characterize critical fish refuge areas and determine the habitat features that will enable species to persist within them.
- Longer-term and larger-scale movement patterns of the larvae of a range of species of fish, as well as the role of reach-scale flow retention and survival of wild and stocked young fish.
- We have demonstrated that an otolith chemistry/isoscape approach has applicability to investigations concerning the spatial structuring of populations and the spatial scale of key life history processes, across a range of scales (selected area–Basin) and species. Knowledge gaps emanating from our EWKR investigations include:
 - A need to bolster water $^{87}\text{Sr}/^{86}\text{Sr}$ data for rivers of the northern MDB to account for temporal variability and strengthen our current dendritic isoscape.
 - Further exploration of Golden Perch recruitment sources and movement in the rivers of the northern Barwon-Darling system.
 - Mechanisms promoting recruitment in regions which appear to be important recruitment sources.
 - Mechanisms inhibiting natural recruitment in rivers, particularly where stocked fish dominate populations
 - Prospective species for future investigation includes silver Perch and Murray Cod, both high priority species for water managers and the general public.
- Examination of the role of predation and competition with non-native species during early-life stages. Whilst these are not factors that are related to flow, they represent a significant source of mortality for the early-life stages of native species
- Examination of food requirements and diet of fish early life stages of a range of species, particularly in northern MDB.
- A greater diversity of species: the work undertaken for the EWKR Fish Theme focused largely on Golden Perch and Murray Cod, the two highest priority species for water managers. There is a need to examine recruitment drivers and the influence of flow for a greater number of species. Of concern are the small-bodied floodplain species, many of which are critically endangered.

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