University of New England

Response of fishes to anthropogenic fragmentation of the Barwon-Darling River

A thesis submitted by

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Declaration

I hereby certify that the content of this thesis has not been and is not being submitted for any other degree to this or any other university. I also certify that the work contained in this dissertation is my own and that al help received in preparing this thesis and all sources used have been duly acknowledged.

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Signature of Candidate Date

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Preface

This thesis is submitted as a thesis by publication. It is written in journal article format with independent chapters intended as publications. The contents of some of the chapters of this thesis have been submitted or are intended to be submitted to journals.

Journal Articles:

Chapter Three

Fish response to anthropogenic fragmentation in rivers: A systematic review. To be submitted to *Oecologia*.

Chapter Four

Fish fins as a non-lethal alternative to muscle tissue in stable isotope studies of food webs in an Australian river. Accepted for publication in *Marine and Freshwater Research*.

Chapter Five

Food web structure of fishes in the Barwon-Darling River. Submitted to *Freshwater Biology*.

Chapter Six

Population structure of bony bream (*Nematalosa erebi*) in the Barwon-Darling River. Submitted to *Freshwater Biology*.

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Summary of Thesis

Dryland rivers are ecologically important freshwater habitats in otherwise dry environments. They drain a substantial proportion of the world's freshwater, yet they have been understudied relative to rivers in temperate climates. Rivers are threatened by water demands for human needs and in arid environments by an increasing scarcity of surface water due to a changing climate. The Barwon-Darling River in the upper Murray-Darling Basin in Australia is a dryland river characterised by extreme hydrological variability. It has experienced changes in hydrology and geomorphology post-European settlement, and reduced abundances of native species. This thesis aims to better understand the impact of water resource development and increased fragmentation on food web structure and genetic connectivity of fishes in the Barwon-Darling River.

A systematic literature review was undertaken to identify the body of literature available to understand the impact of fragmentation on fish in all river types globally. The literature was then used to test specific hypotheses related to food web and genetic responses of fishes to fragmentation. There was strong evidence that the creation of lentic habitat alters food webs, but inconsistent evidence about the effect of hydrological modification on food web structure. There was evidence that some species-specific traits influence the effect of fragmentation on genetic differentiation, but inconsistent evidence that barriers lead to genetic differentiation in general. The literature review highlighted the need for more long-term, interdisciplinary research which includes a range of causes of fragmentation. More comprehensive studies which account for a range of relevant factors influencing the response of fish to fragmentation are needed to help understand the mechanistic link between anthropogenic fragmentation and the biotic response.

The use of stable isotopes in food web studies often relies on invasive or lethal sample collection methods. This has the potential to alter community structure and is undesirable particularly in areas with an already sparse population. This study found a strong isotopic relationship between fin and muscle tissue in three species common to Australian dryland rivers: *Macquaria ambigua, Cyprinus carpio, and Nematalosa erebi*. The results showed that non-lethally collected fin tissue can be used as a proxy for muscle tissue in isotopic trophic studies. Tissue conversion equations were developed to predict muscle $\delta^{13}C$ and $\delta^{15}N$ isotope ratios from fin tissue values. There were significant but consistent differences between fin and muscle $\delta^{13}C$ values for all species, and fin tissue was a good predictor of muscle tissue $\delta^{13}C$ ($r^2 = 0.77$ for all species). The relationship between $\delta^{15}N$ values was less consistent, with a significant difference found in only one species, fin tissue was still a good predictor ($r^2 = 0.72$ for all species). Developing species-specific tissue conversion models resulted in the least amount of error, but regional models result in similar error and are more accurate than

general global models. These results are consistent with findings from prior studies of different species, enabling the use of non-lethally collected fin tissue in stable isotope food web studies.

Food web structure was investigated in six sites in the main channel of the Barwon-Darling River to determine if varying levels of hydrological isolation influenced trophic interactions. Samples were collected in March 2019 during a period of no flows when the river had receded into a series of disconnected waterholes. Analysis was restricted to four species that were caught in high enough abundance at all sites. Stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) were used to determine consumer trophic position and community metrics. There were high levels of omnivory at all sites and indications of spatial variation with some sites showing more trophic overlap. However, no consistent pattern was found between food web structure and site differences with respect to connectivity. Widespread omnivory and short trophic links are expected in variable environments. Explaining spatial variation among sites likely requires a more thorough assessment of food web structure and site variables than this study allowed.

Functional connectivity within the Barwon-Darling River was assessed based on genetic structure of bony bream, *Nematalosa erebi,* a common and widespread species. Samples were collected from eight sites along the length of the main river channel and two remote sites, one from a tributary river and one from near the mouth of the Murray River. Single nucleotide polymorphism (SNP) markers were used to assess if genetic structure is related to anthropogenic fragmentation. There was differentiation among sites, but there was no positive correlation between genetic differentiation and geographic or resistance distance between sites. Anthropogenic fragmentation of the river began about 150 years ago with substantial modification beginning in the 1960s and detecting any genetic response likely requires several more generations. Similar to other species in extinction prone environments, *N. erebi* may rely on metapopulation dynamics to persist in this highly variable river.

The dynamic nature of dryland rivers complicates efforts to understand the response of the biological community to anthropogenic fragmentation. Species that have persisted in these variable environments are adapted to extended periods of natural fragmentation, making any response to altered fragmentation difficult to detect. Overall, there was spatial variation in food web structure and genetic differentiation among sites within the river. However, there were no consistent patterns that would help us understand the impact of fragmentation. This study has helped illustrate that assessing the response of the fish community to water resource development in dryland rivers is complicated and finer-scale assessment may be necessary to detect impacts.

Introduction to fish response to anthropogenic fragmentation of the Barwon-Darling River

1.1 Introduction

Humans rely on rivers for a range of ecosystem services such as transportation, energy, food, and freshwater (Haidvogl, 2018). As a result, rivers have been greatly modified for human needs (Arthington, Naiman, McClain, & Nilsson, 2010; Poff et al., 2003). Rivers face a multitude of threats such as habitat alteration, invasive species, pollution, and eutrophication (Malmqvist & Rundle, 2002) in addition to a changing climate (Palmer et al., 2009). Globally, human activities threaten both the biodiversity of river habitats and water security for human needs (Vörösmarty et al., 2010). Perhaps the greatest threat to biodiversity in rivers is water resource development, which has changed the structure and function of riverine ecosystems (Benke, 1990; Liermann, Nilsson, Robertson, & Ng, 2012; Nilsson, Reidy, Dynesius, & Revenga, 2005). The construction of dams, weirs, levees, and other hydrological alteration fragments habitats, restricts movements, and alters ecosystem processes (Fuller, Doyle, & Strayer, 2015).

The Murray-Darling Basin (MDB) covers approximately 14% of Australia's land area and is a vitally important region for agriculture (Leblanc, Tweed, Van Dijk, & Timbal, 2012). As such, the basin has been heavily modified to meet agricultural needs and faces a number of additional pressures that degrade ecosystem condition including introduced species, fragmentation, overexploitation, and habitat modification (Gawne et al., 2011; Koehn, 2015). The MDB extends across a range of temperature and precipitation gradients, with precipitation decreasing from east to west and varying seasonally north to south (Larkin et al., 2020). The Murray River, which drains the southern portion of the basin, is perennial with large areas of forested floodplain; whereas the Barwon-Darling River, which drains the northern part of the basin, is more hydrologically variable and flows are usually confined within the channel (Walker, 1985). Most of the length of these rivers flows through arid and semi-arid zones west of the Great Dividing Range (Larkin et al., 2020)

Dryland rivers flow through arid or semi-arid regions and have variable flow regimes ranging from predictable seasonal flows to highly intermittent and unpredictable flows (Powell, 2009; Walker et al., 1995). Dryland rivers in Australia are hydro-geomorphologically diverse (Thoms et al. 1996; 2006; Thoms and Sheldon 1997, Pearson et al. 2020; Larkin et al. 2020). These rivers comprise river types ranging from single continuous channels to discontinuous terminating channels (Larkin et al., 2020). Within the MDB, dryland rivers range from continuous with permanent wetlands to discontinuous

and declining with intermittent wetlands, with the northern part of the basin having more discontinuous rivers (Larkin et al., 2020)

Dryland rivers are some of the most degraded in the world (Revenga, Brunner, Henninger, Kassem, & Payne, 2000). Despite these ongoing changes, there is still a lot to learn about how dryland rivers function naturally (Tooth, 2000). The effects of anthropogenic drivers, such as flow alteration, on the biological community are highly variable (Poff & Zimmerman, 2010). Much of the existing research on ecosystem response to anthropogenic changes has focused on temperate rivers (see chapter three) which have predictable variability in hydrology linked to seasons (Junk, Bayley, & Sparks, 1989; Vannote, Minshall, Cummins, Sedell, & Cushing, 1980). Responses of fish to changes in flow is context dependent and varies based on attributes of the flow event, the habitat, and the fish species (Walters, 2016). Understanding responses to anthropogenic change relies on studying them in context, and the bias in research toward temperate rivers means even less is known about dryland rivers where the natural flow regime is often intermittent and unpredictable (Powell, 2009; Walker, Sheldon, & Puckridge, 1995).

This thesis aims to investigate how anthropogenic fragmentation of a highly variable and unpredictable dryland river ecosystem has influenced the biological community. By aiming to improve our understanding of dryland rivers, it addresses an important knowledge gap. This research uses food web structure as an indicator of fish community interactions within habitats of a dryland river, and genetic structure of a widespread fish as an indicator of functional connectivity within the river. These two response variables integrate different temporal scales. Food web structure reveals recent patterns, while genetic structure reflects longer-term patterns and processes.

1.2 Dryland river ecology

All rivers exhibit natural flow variability (Poff et al., 1997; Walker, 1985), but it is particularly pronounced in dryland rivers, which experience some of the highest levels of hydrological variability and unpredictability (Puckridge, Sheldon, Walker, & Boulton, 1998). This variable and unpredictable hydrological connectivity is an integral part of ecosystem function within many dryland rivers (Sheldon, Boulton, & Puckridge, 2002; Walker et al., 1995), and may produce uniquely adapted biological communities (Amoros & Bornette, 2002; Lytle & Poff, 2004). Intermittent dryland rivers in Australia are often described as having ‘boom-and-bust’ ecology, where floods initiate productivity and habitat connectivity (‘boom’ period) followed by disconnection and survival during drought

('bust' period) (e.g. Kingsford & Johnson, 1998; Puckridge et al., 1998; Puckridge, Walker, & Costelloe, 2000).

In intermittent dryland rivers, periods of high flow and connectivity to the floodplain promote the transfer of nutrients and biota, among other things, throughout the river (McGinness & Arthur, 2011; Thoms & Sheldon, 2000). High flows function as cues for biota to leave waterholes (Marshall et al., 2016; Puckridge et al., 2000), exploit resources on the floodplain (Bunn, Thoms, Hamilton, & Capon, 2006), and colonise new areas (Bond et al., 2015). In rivers with naturally high turbidity, stable water levels create a narrow littoral zone for primary production by algae (Bunn et al., 2006), sustaining the biological community during dry periods. Fish are able to persist during low production periods by having generalist feeding habits and shifting their diets based on productivity (Leigh, Burford, Sheldon, & Bunn, 2010; Sternberg, Balcombe, Marshall, & Lobegeiger, 2008). During periods of low flow and disconnection, waterholes act as important habitat refugia (Arthington, Balcombe, Wilson, Thoms, & Marshall, 2005).

The Barwon-Darling River is a semi-arid river system in south-eastern Australia and serves as an example of an intermittent dryland river. It has a catchment area of 699,000 km^2, 60% of which is less than 300 m above sea level, and a main channel length of 2,700 km (Webb Mckeown, 2007). The Barwon-Darling River has one of the most variable flow regimes in the world (Puckridge et al., 1998), existing for most of the time in a low flow stage when water is restricted to the main channel (Thoms, Sheldon, & Crabb, 2004). Surface water in the main channel contracts to disconnected waterholes during periods of extended drought (Walker et al., 1995).

1.3 Anthropogenic fragmentation in the Barwon-Darling River

Intermittent dryland rivers, by nature of their highly variable flow regime, exhibit enhanced natural fragmentation. The ecological community is influenced by long term drivers, with organisms in dryland rivers adapted to environmental variability and heterogeneity (Lytle & Poff, 2004). The variable and unpredictable disturbance regime and alteration between 'boom' and 'bust' periods maintains these populations and their habitat requirements (Bunn et al., 2006). Water resource development alters this natural variability and organisms' responses to it. In-channel weirs stabilise water levels and habitat while disrupting fish movement (Baumgartner, Zampatti, Jones, Stuart, & Mallen-Cooper, 2014; Pearson et al., 2020). Water storage and diversion has altered flow variability (Mallen-Cooper & Zampatti, 2020), potentially interrupting important life-history cues such as fish movement amongst habitat patches (Marshall et al., 2016). An improved understanding of the

response of the biological community to anthropogenic fragmentation is necessary to inform management decisions in the face of this unprecedented environmental change.

Anthropogenic fragmentation of the Barwon-Darling River started in the 1800s with the oldest weir opened in 1897, but extensive modification began in the 1960's in the form of dams, weirs, and water abstraction (NSW DPI, 2019). Water resource development has altered the natural hydrology and geomorphology of the river. Post-regulation of the river, the magnitude of near-annual flow pulses has been reduced by over 90%, resulting in longer durations of lentic conditions while the spatial extent of lotic conditions has been limited during droughts due to weir pools (Mallen-Cooper & Zampatti, 2020). A change in hydrological character was most evident in low-flow periods post-2000. In general, sections of the river upstream from the town of Bourke have experienced more frequent, but shorter, periods with flows low enough to disconnect habitats, while sections downstream of Bourke experience fewer total dry spells, but the duration of dry spells is longer (MDBA, 2018). The physical template of the river has also changed since European settlement in the mid-1800s. The depth of waterholes within the influence of weirs has increased and the distance between waterholes has decreased, but outside weir influence depth has decreased and distance has increased (Pearson, Reid, Miller, & Ryder, 2020). Additionally, river regulation has altered the complexity of the channel by reducing horizontal 'benches' that play an important role in organic matter retention (Sheldon & Thoms, 2006). Thus, habitat within the main channel has changed along with the hydrological connectivity among those habitats.

Altered hydrology and geomorphology of the river has also coincided with changes in the fish community. In regulated reaches of the Barwon-Darling River, which have an increased stability of river flows and a reduced frequency of natural disturbances, there was a reduced abundance of native species compared to unregulated reaches (Gehrke, 1997). Increased river regulation and the desynchronising of environmental cycles and reproductive cycles of native fish also corresponded to a reduction in species diversity and an increase in non-native species (Gehrke, Brown, Schiller, Moffatt, & Bruce, 1995). It is estimated that native fish populations are only about 10% of their pre-European settlement levels, and biomass in the Murray-Darling Basin is dominated by non-native invasive species (MDBA, 2004).

1.4 Indicators of ecological responses to anthropogenic disturbance

The range of potential indicators to measure ecological responses to fragmentation is extensive. Fishes were chosen as a subject because they are relatively long-lived, are higher order consumers, encompass a range of movement and life-history strategies, and thus should reflect the effect of fragmentation at a range of spatial and temporal scales. Additionally, studies have shown that fish

respond to flow alteration (Poff & Zimmerman, 2010; Webb et al., 2010). Many studies assessing ecological responses to disturbance focus on structural responses such as changes in species assemblage or diversity (Poff & Zimmerman, 2010; Leigh, Stewart-Koster, Sheldon, & Burford, 2012), rather than indicators of ecosystem function such as rates primary production. This study aimed to focus on functional ecosystem measures that have not been well-studied within the Barwon-Darling River.

Food webs and genetic structure were selected as functional indicators because they have been well-studied in general and are likely to be influenced by different spatial and temporal scales of fragmentation. Food webs reflect species interactions and energy pathways within the biotic community (Cross et al., 2013; Vander Zanden, Chandra, Allen, Reuter, & Goldman, 2003) and patterns of genetic structure are effective indicators of functional connectivity, or the ability of a species to disperse and re-populate areas (Tischendorf and Fahrig 2000). These attributes make food webs and genetic structure ideal features of ecosystems to examine when exploring the role of connectivity as a driver of ecosystem structure and function.

1.5 Food webs in dryland rivers

Food webs provide a tool to view fundamental ecosystem processes such as species interactions and energy pathways within the biotic community (Cross et al., 2013; Vander Zanden, Chandra, Allen, Reuter, & Goldman, 2003). They reflect community interactions and can help elucidate the structure and functioning of an ecosystem (Thompson et al., 2012). Food webs themselves are complex systems influenced by a myriad of factors (Power & Dietrich, 2002), such as hydrological regime, river geomorphology, and water quality (Robson et al., 2017). They are shaped by a range of processes operating at different spatial and temporal scales, where landscape processes such as movement of organisms within the river network influences species composition and thus interactions within small habitat patches (Woodward & Hildrew, 2002).

Food webs in hydrologically variable rivers, such as intermittent dryland rivers, are characterised by widespread omnivory and short trophic links (Blanchette, Davis, Jardine, & Pearson, 2014; Douglas, Bunn, & Davies, 2005; Pusey, Arthington, Stewart-Koster, Kennard, & Read, 2010). These food web features likely arise because unpredictable food resource availability discourages dietary specialisation, and species have adapted with dietary flexibility. Opportunism and omnivory may confer ‘dynamic stability’ to the food web that provides a buffer against flow extremes (Leigh et al., 2010). These adaptations combined with low species diversity suggests that there will be minimal

spatial variation in food web structure. However, studies in other variable environments have found spatial and temporal variation related to frequency of hydrological connection (Reid, Delong, & Thoms, 2012) and environmental conditions (Blanchette et al., 2014).

1.6 Genetic connectivity in dryland rivers

Genetic markers provide a tool for assessing functional connectivity given that connected populations will share similar allele frequencies, and populations that are disconnected will differ in allele frequencies (Slatkin, 1993). Genetic differentiation reflects decreased connectivity between local populations over time. Landscape genetics provides a framework for understanding how landscape features and fragmentation have influenced genetic structure by combining concepts from landscape ecology with genetic analysis (Anderson et al., 2010; Grummer et al., 2019; Manel, Schwartz, Luikart, & Taberlet, 2003).

Species traits influence the genetic response to landscape changes and species with strong dispersal ability may be able to overcome barriers to movement and maintain genetic connectivity in fragmented habitats (Faulks, Gilligan, & Beheregaray, 2010). Within the Murray-Darling Basin, widespread species with strong dispersal ability show moderate levels of phylogeographic structure (Unmack, 2013) and patterns of genetic structure that suggest strong connectivity across large spatial scales (Attard et al., 2018; Bostock, Adams, Laurenson, & Austin, 2006; Harrisson et al., 2017). However, widespread smaller-bodied species with limited dispersal potential exhibit reduced genetic connectivity related to recent habitat fragmentation (Cole et al., 2016; Lean et al., 2017).

The ideal indicator species to assess the impact of fragmentation on genetic connectivity would have traits that make it susceptible to the effects of fragmentation such as low mobility (Ewers & Didham, 2006; Lean et al., 2017). However, despite the use of effective and thorough sampling techniques, low numbers of fish were collected and only four species were caught consistently at all sites. This restricted species selection to only two native species that had reasonable sample sizes: golden perch and bony bream. Ultimately, bony bream was selected as it provided the largest sample sizes within the greatest range of sites. Genetic structure of golden perch throughout the MDB has been well-studied and was used as a comparison for genetic structure of bony bream. While not an ideal indicator of the effects of fragmentation, understanding of the current genetic structure will aid in our understanding population dynamics and identification of any future changes within the river

1.7 Thesis aims

This thesis aims to investigate how anthropogenic fragmentation of a highly variable dryland river ecosystem has influenced the biological community. The Barwon-Darling River naturally has highly variable hydrology which has been altered due to water resource development and has led to increased physical fragmentation. The three main objectives of this research were to:

1. identify gaps in the research on how fish are influenced by anthropogenic fragmentation in rivers, specifically regarding functional responses of food web dynamics and genetics;
2. determine if spatial variation in food web structure in the main channel of the Barwon-Darling is associated with the effects of water resource development;
3. assess if anthropogenic fragmentation in the Barwon-Darling River is reflected in genetic structure of *Nematalosa erebi*, a widespread and common fish.

I expected that effects of anthropogenic fragmentation would be difficult to detect due to adaptations of native fish to the variable flow regime. However, I predicted some consistent patterns in food web structure that would reflect differences in hydrological connectivity of habitats, and patterns in genetic markers associated with habitat connectivity.

1.8 Thesis structure

Chapter two provides a review of the literature used to understand the theoretical concepts used to design the study and answer the research questions.

Chapter three is a systematic literature review used to identify the body of literature on the specific issue of fish community response to fragmentation and an assessment of the strength of evidence available to understand how anthropogenic fragmentation has influenced food web dynamics and genetic structure. This chapter is written as a standalone manuscript that will be submitted to *Oecologia*.

Chapter four establishes the utility of using non-lethally collected fish tissue as a surrogate for lethally collected tissue in isotopic studies of food webs. This chapter is a standalone manuscript that has been published in the journal *Marine and Freshwater Research*.

Chapter five compares the fish-centred food web structure across six sites within the main channel of the Barwon-Darling River. This chapter is a standalone manuscript that has been submitted for publication to *Freshwater Biology*.

Chapter six investigates if anthropogenic fragmentation of the Barwon-Darling River has influenced genetic structure of *Nematalosa erebi*. This chapter is a standalone manuscript that has been submitted for publication to *Freshwater Biology*.

The final chapter summarises and synthesises the findings of the research making up this thesis.

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Literature Review

This literature review focuses on the general concepts guiding this research. It provides an overview on viewing rivers as complex adaptive systems, principles of landscape ecology as applied to rivers, and some background on connectivity and fragmentation. The more specific aspects of the effects of fragmentation on river ecosystems and associated knowledge gaps are addressed in the structured literature review in the next chapter.

2.1 Rivers as complex adaptive systems

River ecosystems can be viewed through the lens of complex adaptive systems. Although there is no strict definition of complex adaptive systems (CAS), there are certain elements common to all CAS. Three of those elements as noted by Levin (1998) are: (1) they have sustained diversity and individuality of the components; (2) there are localised interactions among the components; and (3) there is an autonomous process that selects from among those components a subset for replication or enhancement (self-emergence). In CAS, hierarchies naturally emerge from patterns formed by local interactions within the system (Levin and Segel 1985), and cannot be imposed upon the system. Systems that exhibit these elements will have the ability to change composition in response to selective processes, and continuously move towards a community that is best suited to those selective processes (Norberg 2004).

The elements of complex adaptive systems, as applied to biological systems, are termed biocomplexity (Norberg 2004). Biocomplexity can be defined as "the degree to which the interactions in ecological systems comprising biological, social, and physical components incorporate spatially explicit structure, organizational connectivity, and historical contingency" (Pickett et al. 2005). Cadenasso et al (2006) provide a framework for biocomplexity in coupled human-natural systems to help explain how complexity applies to biological systems. The three dimensions of complexity in this framework are: heterogeneity, connectivity, and contingency. The heterogeneity dimension refers to how discrete areas that differ from one another (i.e. patches) are organized. Note that increasing spatial scale increases patch complexity. Connectivity explains the organisation of interactions within and between patches. Interactions within a habitat patch influence interaction between and among patches. Contingency describes the temporal influence on direct and indirect links between patches. Legacies and lagged effects influence contemporary links. Complexity increases up the hierarchies of these dimensions as more processes must be accounted for.

The complexity of a river is driven, in part, by its flow regime (Walker et al. 1995). Degree of complexity is influenced by variability in the flow regime which determines patch heterogeneity, connectivity, and contingency history. Recognizing the hierarchical scale at which a system is being observed aids in understanding the biocomplexity by accounting for the various processes, operating across scales, that contribute to that complexity.

Rivers exhibit many of the characteristics of a complex adaptive system. They exhibit aggregation in that they are organised as nested hierarchies (Burke et al. 2009, Frissell et al. 1986, Parsons et al. 2004) and these levels of organisation allow characteristic properties to emerge when viewed at appropriate scales (Parsons and Thoms 2007). Scales at one level influence, or are influenced by, levels above and below it (Malmqvist 2002). Rivers are nonlinear systems driven by variable flow regimes and periodic disturbances (Poff et al. 1997) which create unpredictability in time and space. Hydrology (the flow regime) facilitates connectivity among components of the river system. Diversity is maintained by the movement of species within the river system (Morrissey and de Kerckhove 2009), and the maintenance of this diversity allows the ecosystem to adapt to the loss of a species. These processes create spatiotemporal heterogeneity which results in biocomplexity (Hohensinner et al. 2014, Thorp et al. 2006, Ward et al. 1999).

Complex adaptive systems cannot be reduced to the sum of their parts and studying these systems in an unintegrated way can produce misleading results (Levin et al. 2013). Recognizing rivers as complex adaptive systems allows for a better understanding of the characteristics that allow rivers to adapt and evolve. Perhaps most importantly how connectivity between components creates the system.

2.2 Landscape ecology

The field of landscape ecology is concerned with spatial patterns and ecological processes across multiple scales (Turner 1989). More specifically, the study of landscape ecology considers: the development and dynamics of spatial heterogeneity, spatial and temporal interactions and exchanges across heterogeneous landscapes, influences of spatial heterogeneity on biotic and abiotic processes, and management of spatial heterogeneity (Risser and Karr 1984).

From the terrestrial perspective, landscapes are composed of three components: patches, corridors, and the matrix (Forman 1995). A patch is ‘a homogenous area that differs from its surroundings’. Corridors are ‘strips that differ from their surroundings’ and can act as barriers, habitat, or conduits. Matrix underlies the patches and corridors, and acts as the background that controls landscape

dynamics. The patch-corridor-matrix mosaic creates the heterogeneous landscape (Forman 1995). Spatial arrangement of patches and corridors within the matrix determines connectivity and movement of organisms and matter between patches (Turner 1989), which influences the structure and function of landscapes.

Structure is the spatial distribution of energy, materials, and organisms, which vary in relation to the size, shape, type, and configuration of components (Noss 1990). Function refers to the interactions among patches in terms of the flow of energy, materials, and organisms (Noss 1990). Change is the alteration of structure and/or function of landscape components. Structure, function, and change of landscapes are scale-dependent (Turner 1989). Scales can be defined in terms of grain and extent. Grain is the smallest scale of homogenous spatial resolution (patch); and extent is largest scale of heterogeneity that an organism responds to (Kotliar and Wiens 1990), or scales at which processes occur. Importantly, homogeneity, and heterogeneity, is determined by an organism's perception of its environment and varies among species (Kolasa 1989, Pringle et al. 1988).

Landscapes can be viewed as hierarchically nested mosaics (Forman 1995). Levels within the hierarchy consist of multiple levels of patchiness (i.e. the second level contains multiple first levels (grain), and the third level contains multiple second levels which contain multiple first levels), the number of levels in the extent of the hierarchy is determined by the organism or question of interest (Kotliar and Wiens 1990). Defining the scale of a study is important as a process or pattern may be important at one scale but insignificant at another (Turner 1989) due to interactions within the hierarchy.

Hierarchy theory dictates that different levels of organisation (scales) have functional linkages between them, with each level being influenced by or exerting influence on levels below and above it (O'Neill et al. 1989). Adjacent levels in the hierarchy will have a larger influence on the levels immediately surrounding it than levels farther away (Kotliar and Wiens 1990). Small scales contain lower levels of organisation which are governed by smaller scale processes and higher levels of organisation constrain organisation of lower levels by events that are large and slow (Urban et al. 1987).

Hierarchical patch dynamics (Wu and Loucks 1995) brings together patch dynamics and hierarchy theory. It views ecosystems as nested hierarchies of patch mosaics and provides a theoretical framework for linking pattern with process at different scales. It addresses connectivity, disturbance, heterogeneity, and the multiple spatial and temporal scales at which landscape processes operate. A change in the lower level of a hierarchy will, over time, be reflected in the higher levels (Wu and Loucks 1995).

The basic concepts of landscape ecology are useful for understanding the structure and function of landscapes across large geographic areas. Patches and their organisation within a nested hierarchy help break down the large-scale focus of landscape ecology into patterns and process at a variety of scales. Recognising the influences and constraints imposed by levels of the hierarchy is vital to make sense of the complexity of a system. Approaching the study of rivers as complex adaptive systems requires explicitly studying a process at the appropriate scale for the response of interest.

2.3 Riverine landscapes

Rivers have been viewed as a nested hierarchy (Burke et al. 2009, Frissell et al. 1986, Parsons et al. 2004), which can exhibit high levels of spatio-temporal complexity within each level of the hierarchy (Ward et al. 2002). Applying the basic tenets of landscape ecology, and its broad geographic scope to assessing pattern and process, provides a framework to better understand the complexity of river systems. In terrestrial ecology, landscapes are composed of patches, corridors, and matrix (Forman 1995), with rivers acting as corridors within the terrestrial matrix. When applying these concepts to rivers, the river exists as a separate landscape and not an element within the terrestrial landscape.

The central theme of landscape ecology is the influence of spatial patterns on ecological process (Turner 1989). Wiens (2002) outlined how concepts of landscape ecology can be applied to riverine landscapes. The key landscape ecology concepts, identified by Wiens (2002) were: (1) Patches differ in quality – patches contain different quality habitat, change over time with flow, and organisms respond to these variations. (2) Patch boundaries affect flow – what happens in a patch is a function of the exchanges (or flow) across boundaries. The permeability of boundaries is dynamic and changes with flow. (3) Patch context matters – the composition and context of the boundary will have an influence on internal patch properties and patch interactions. (4) Connectivity is critical – patch quality, boundary, context, and spatial configuration of patches all determine connectivity. Water-flow pathways (corridors) are dynamic and temporally variable. (5) Organisms are important – different organisms have varying responses to the heterogeneous structure of landscapes, and patches and boundaries are organism specific. (6) The importance of scale – the processes that produce habitat patches and responses of organisms to those patches are scale dependent.

The variable permeability of patch boundaries (or matrix) is an important determinate of connectivity among habitat patches (Wiens 2002). Patches are defined based on the habitat requirements of the organism of interest (Pringle et al. 1988), and the matrix can be defined as areas that do not meet those habitat requirements, thus forming the boundary of that habitat patch. The

matrix is selectively permeable dependent on how inhospitable the conditions are for the organism of interest, and the permeability can vary temporally with flow and changes in abiotic conditions (Erős and Campbell Grant 2015). Resources within, and permeability of, the matrix influence within patch dynamics (Wiens 2002). Connectivity between habitat patches, and area of matrix habitat is largely a function of flow.

The spatiotemporal organisation of river systems as nested hierarchies and the interdisciplinary nature of river science necessitates a clear understanding of the effect of scale when assessing patterns and process (Parsons and Thoms 2007). The appropriate scale is relative to what is being investigated (Dollar et al. 2007), and the patterns and processes that are detected are determined by the scale at which they were measured (Wiens 1989). The three subsystems of river science, geomorphology, hydrology, and ecology, each have their own hierarchies. These hierarchies interact with each other at different scales and contribute to the biocomplexity of rivers (Dollar et al. 2007, Thorp et al. 2008). The appropriate spatial and temporal scales are necessary to detect the biological response to a change in the system. High frequency biological process such as behaviour or physiological changes necessitate assessment at smaller spatial and temporal scale; whereas lower frequency processes like gene flow or physical habitat change that are influenced by the whole hierarchy require assessment at larger scales (Anderson et al. 2010, Poff and Ward 1990). A hierarchical framework provides a way of organising multiple scales of measurement (Dollar et al. 2007, Parsons et al. 2004) and incorporating the complexity inherent within each scale into our understanding of the biological response of interest.

2.4 Connectivity in rivers

In riverine landscapes, flow regime is the key driver of connectivity (Wiens 2002). Hydrologic connectivity can be defined as the 'water-mediated transfer of matter, energy, or organisms within or between elements of the hydrologic cycle' (Pringle 2001). Connectivity is driven by the natural flow regime, which can be described based on five components outlined by Poff et al (1997): (1) *magnitude* of discharge is the amount of water flowing past a fixed point; (2) *frequency* of occurrence is the return period of a flow above a certain magnitude; (3) *duration* is the period of time a flow event persists; (4) *timing*, or predictability, of flows of a defined magnitude is the regularity with which they occur; (5) rate of change, *flashiness*, is how quickly flow shifts from one magnitude to another. The interaction between the natural flow regime and local geology influences the type and distribution of physical habitat patches (Frissell et al. 1986). This interaction, in turn, acts to determine the biotic composition within a given river (Bunn and Arthington 2002).

The flow regime and its influence on physical habitat and ecological processes operate on a hierarchical scale. Long term (large temporal scale) variation of the flow regime defines river geomorphology and community composition at a large spatial extent; whereas short term changes (small temporal scale) in flow and local hydraulics influence habitat availability and processes like nutrient cycling at small spatial scales (Biggs et al. 2005). The interactive nature of hydrology, geomorphology, and biology at various scales creates a dynamic and heterogeneous environment. Varying levels of hydrological connectivity and geomorphological dynamics create heterogeneous habitat patches (Amoros and Bornette 2002), structuring biological communities such as fish assemblages (Zeug et al. 2005) and macrophytes (Sousa et al. 2011). Ultimately, these physical processes may promote both biodiversity (Sheldon et al. 2010) and stability (Bellmore et al. 2015).

Hydrologic connectivity of a river system occurs at multiple spatial and temporal scales, and in four dimensions. The four dimensions of lotic connectivity as laid out by Ward (1989) are: longitudinal, lateral, vertical, and temporal. The longitudinal dimension encompasses the upstream and downstream linkages within the channel. Lateral connectivity occurs between the main channel and the floodplain. Vertical connectivity is the interaction between the surface water, the hyporheic zone, and the ground water. The temporal dimension can refer to the timing, duration, frequency, recurrence interval, or rate of change in connection.

Flow is the driver of interactions among patches that define ecosystem structure and function in rivers (Poff et al. 1997). Hydrologic connectivity across all four dimensions is a critical component of riverine landscapes. Connectivity among habitat patches drives ecosystem structure (Amoros and Bornette 2002), is important for organic matter and nutrient exchange (Tockner et al. 1999), and facilitates the movement of organisms between habitats (Zeug and Winemiller 2008). Structural connectivity of a landscape is a measure of habitat contiguity and can be assessed based on landscape structure. Functional connectivity, on the other hand, is a measure of an organism's behavioural response to various elements within a landscape (e.g. patches and boundaries; (Tischendorf and Fahrig 2000). For example, patches in a river may be structurally connected by water, but may not be functionally connected from a rheophilic fish's perspective if they are required to move through a reservoir to reach the next patch of flowing water (Clarke et al. 2007).

2.5 Fragmentation in rivers

Habitat fragmentation in terrestrial landscapes is described as a process that occurs when 'a large expanse of habitat is transformed into a number of smaller patches of smaller total area isolated

from each other by a matrix of smaller patches of smaller total area, isolated from each other by a matrix of habitats unlike the original' (Wilcove et al. 1986). Fragmentation of habitat results in a change in patch number, size, connectivity, or isolation (Forman 1995). From a species' perspective, fragmentation and habitat loss can be caused by habitat degradation, the gradual deterioration of habitat quality, and habitat sub-division, the breaking apart of continuous habitat into multiple patches (Fischer and Lindenmayer 2007). Habitat sub-division can lead to an increase in patch isolation and alter species interactions (Fischer and Lindenmayer 2007).

In riverine landscapes, patches are not discrete, and fragmentation is more dynamic. Natural fragmentation in river systems (i.e. disconnection) is a function of natural hydrology in which the degree of fragmentation changes frequently with discharge (Poff et al. 1997). Natural variability of the flow regime, including disconnection, plays an integral role in shaping river ecosystems (Biggs et al. 2005, Bunn et al. 2006, Greet et al. 2011, Sheldon and Thoms 2006). However, in rivers with altered physical templates, anthropogenic influences also become drivers of fragmentation even when flow is not disconnected (Fuller et al. 2015). Alteration of the flow regime or introducing a physical barrier changes the complexity of patches, resulting in a change in the number of habitat patches, their spatial organisation, or the diversity of patches available to meet various habitat requirements (Dunning et al. 1992). Fragmentation can cause both patch isolation due to blocking the flow-pathway (corridor), and increased connectivity due to the stabilisation of flow or creation of unfavourable habitat (increase in matrix habitat).

There are three general types of fragmentation in river networks: habitat, biological, and physical (Fuller et al. 2015). Habitat fragmentation is the result of physicochemical conditions that discourage or prevent movement of organisms. For example, creation of hypoxic zones (Branco et al. 2016) and changes in salinity levels (Wedderburn et al. 2008) can create a chemical barrier to fish movement. Biological fragmentation occurs when there are dense populations of predators, competitors, diseases, etc. that discourage movement. The introduction of a non-native predator to a river can isolate prey populations that are unable to pass through the predator's territory (O'Malley et al. 2013), biologically fragmenting populations. Physical fragmentation is a physical barrier to movement, including lack of water as a medium for movement. Only physical fragmentation will be further explored.

2.6 Potential sources of physical fragmentation

Sources of physical fragmentation can be divided into the two broad categories of natural or anthropogenic. Natural fragmentation is the result of natural river processes and is an important component of the natural flow regime (Biggs et al. 2005). Anthropogenic fragmentation is caused by structures placed in the river, or manipulation of water flow. There are three main agents of natural fragmentation: waterfalls, natural dams, and natural flow variability. Natural fragmentation influences ecosystems in numerous ways and on multiple scales. For example, waterfalls fragment the longitudinal dimension. They can create distinct genetic subpopulations above and below the waterfall (D'Amelio and Wilson 2008, Kelson et al. 2015), create spatial variation in fish assemblages (Barbosa et al. 2015, Silva et al. 2016, Torrente-Vilara et al. 2011), and promote biodiversity on long time scales (Dias et al. 2013). Natural dams, created by beavers or debris accumulation, fragment the longitudinal or lateral dimension. They can increase habitat heterogeneity which increases diversity and abundance of fish in temperate rivers (Smith and Mather 2013), but may also increase non-native fish population in some environments (Gibson et al. 2015). Natural flow variability and drought creates fragmentation of all dimensions, which influences the biological community. For example, over time drought can decrease the diversity of fish assemblages in refuge pools due to habitat isolation (Love et al. 2008). On the other hand, increased isolation of floodplain habitats can increase fish diversity due to less disturbance (Schomaker and Wolter 2011).

Agents of anthropogenic fragmentation vary in degrees of permeability (Puth and Wilson 2001), with dams and weirs generally being the most impermeable. Both cause fragmentation of the longitudinal, lateral, and temporal dimensions. Dams disrupt passage of fish, reducing gene flow among reservoir-fragmented fish populations (Franssen 2012), causing genetic differentiation (Crookes and Shaw 2016), preventing migration (Morita and Yamamoto 2002, Winston et al. 1991), and leading to a simplified food web (Cross et al. 2013). They can also alter flow, creating unsuitable conditions for spawning (Freeman et al. 2001), increasing densities of smaller fish while decreasing density of large-bodied fish (Miranda et al. 2005), and exposing benthos which affects recovery period once flow resumes (Blinn et al. 1995).

Other physical barriers also influence river ecosystems. For example, culverts are permeable but may create an unsuitable corridor and restrict fish movement (Mariano et al. 2012, Warren and Pardew 1998). Levees separate the main channel from the floodplain and are variably permeable based on river water levels. Levees isolate floodplain habitats, reducing water quality and lowering fish species richness, diversity, and evenness (Crites et al. 2012). Isolation reduces gene flow

(Sterling et al. 2012), and degree of isolation can shift fish to more lacustrine adapted assemblages (Dembkowski and Miranda 2011).

In addition to regulating flow with physical structures, water withdrawal for consumptive use can reduce or alter hydrological connectivity. Decreasing water levels can restrict fish movement and affect survival (Fischer and Kummer 2000). Distortion of the natural flow through regulation alters fish life history cues and reduces fish density (Espinola et al. 2014). Alteration of flow can also induce unsteady hyporheic exchange (Hucks Sawyer et al. 2009).

2.7 Summary of Literature Review

Rivers exhibit many of the properties of complex adaptive systems, primarily aggregation, nonlinearity, diversity, and flow, which allow them to adapt and evolve. These same properties mean that the components of rivers cannot be simplified and must be studied in the context of their complexity. The basic concepts of landscape ecology as applied to rivers provide a framework for organising the complexity of these large systems, particularly the concepts of scale and hierarchy. An understanding of the spatial and temporal scale at which a process occurs, and the corresponding scale of influential processes, breaks down the complexity of the river system.

Hydrology shapes the physical habitat and biological community of the river. Changes in natural hydrology affect connectivity among habitat patches and influence the pattern and process of rivers. Although hydrological disconnection is a natural component of the flow regime, anthropogenic fragmentation creates different selective pressures on the biological community, driving fundamental changes to community structure and function.

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Fish response to anthropogenic fragmentation in rivers: A systematic review

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3.1 Abstract

Anthropogenic fragmentation has changed the structure and function of river ecosystems, but there are still significant gaps in our knowledge. This study used a systematic review to identify the body of literature available to understand the impact of fragmentation on fishes. That literature was then used to test specific hypotheses related to food web and genetic responses of fishes to fragmentation. There was strong evidence that the creation of lentic habitat alters food webs, but inconsistent evidence about the effect of hydrological modification on food web structure. There was evidence that some species-specific traits influence the effect of fragmentation on genetic differentiation, but inconsistent evidence that barriers lead to genetic differentiation in general. The literature review highlighted the need for more long-term, interdisciplinary research which includes a range of causes of fragmentation. Assessing the literature available to test specific hypotheses revealed that many studies did not quantify the change that resulted from fragmentation. More directed studies accounting for important factors which influence the response to fragmentation are needed to help understand the mechanistic link between anthropogenic fragmentation and the biotic response.

3.2 Introduction

Water resource development has improved water security for human needs, but has changed the structure and function of riverine ecosystems (Benke 1990; Liermann et al. 2012; Nilsson et al. 2005). One of the most significant impacts of water resource development is the increased fragmentation of river ecosystems due to structures such as dams and weirs, or other hydrological alteration (Nilsson et al. 2005). Understanding how water resource development and fragmentation influence ecosystem function is increasingly important to ensure ecological requirements are being met (Naiman et al. 2008), particularly in areas of water scarcity. Identifying areas where more research is needed is a step towards improving this knowledge and mitigating impacts.

The response of river systems to anthropogenic fragmentation and changes in hydrological connectivity has been the focus of river science for decades (Liermann et al. 2012; Nilsson and Dynesius 1994; Pringle 2003; Ward and Stanford 1983). Despite a basic understanding of how fragmentation influences river ecology (Fuller et al. 2015), there are still significant gaps in our knowledge of how changing hydrology and disconnecting habitats affects biotic responses (Miller et al. 2018; Petts et al. 2006; Poff and Zimmerman 2010). Particularly in regard to long term trends (Lindenmayer et al. 2012).

Fragmentation is a component of naturally variable flow regimes and periodic disturbances play an integral role in shaping river ecosystems (Biggs et al. 2005; Bunn et al. 2006; Greet et al. 2011; Sheldon and Thoms 2006). The scale of fragmentation and connectivity can change frequently with discharge (Poff et al. 1997) and biotic responses to fragmentation vary based on the organism of interest (Puth and Wilson 2001; Wiens 2002). Flooding, for example, decreases fragmentation, connecting rivers to their floodplains at varying frequencies with the disturbance creating heterogeneous habitat patches (Amoros and Bornette 2002). While drought increases fragmentation, restricting access to habitat and resources (Lennox et al. 2019). This natural variability results in communities with adaptive strategies allowing them to cope with and even use this variability to their advantage (Lytle and Poff 2004).

Natural fragmentation as a process is inherently dynamic and the complexity of rivers makes this process challenging to understand. Human-induced fragmentation through water resource development adds an additional layer of complexity by altering the spatial patterns and temporal dynamics of this natural process (Horne et al. 2019; Leigh et al. 2012). Physical barriers such as dams and levees, for example, can increase both fragmentation and connectivity by stabilizing water levels above the barrier but restricting movement across the barrier (Pearson et al. 2020). Physical barriers can also be selectively permeable based on water level, creating an impassable barrier during periods of low flow, but allowing movement of organisms during periods of high flow (Baumgartner et al. 2014); or can be selectively permeable based on movement ability of the organism (Bourne et al. 2011). Anthropogenic fragmentation can have multiple impacts. A barrier such as a dam stabilizes flows through impoundment, altering nutrient resources which is reflected throughout the food web (Cross et al. 2013). It also can restrict movement across the barrier preventing connectivity among habitats and changing species' distributions (Liermann et al. 2012), which can also influence food web interactions (Mor et al. 2018).

Another challenge of understanding the effects of fragmentation on rivers is the variability amongst rivers themselves. The flow regime varies between rivers, and components of the flow regime regulate many ecological processes (Poff et al. 1997). Hydrological variability, for example, is linked to climate and run-off, with tropical rivers generally exhibiting low variability and dryland rivers generally exhibiting extreme variability (Puckridge et al. 1998). Flow variability can be reflected in the biological community (Poff and Allan 1995) and it is reasonable to expect different biological responses to flow alteration and fragmentation because of species' adaptations to historic flow regimes (Bunn and Arthington 2002; Poff 2018; Poff and Allan 1995). Ecological responses to flow alteration are not directly transferable across species and geographic setting (Lytle and Poff 2004;

Poff and Zimmerman 2010) and understanding how water resource development and fragmentation influence ecosystem function requires studying a range of rivers and responses.

The aim of this paper was to identify where more research is needed to aid our understanding of the fish response to physical fragmentation of rivers. Fish are relatively long-lived, are higher order consumers, encompass a range of movement and life-history strategies, and should reflect the effect of fragmentation at a range of spatial and temporal scales. There were two primary objectives to understand fish response to physical fragmentation of rivers. First was to use a systematic literature review to identify the body of literature available to answer the question. Second was to evaluate the strength of the existing empirical evidence available to establish a causal link between anthropogenic fragmentation of rivers and fish response to fragmentation. Specifically, fish response based on two functional indicators: food web dynamics and genetic structure of fish populations. The range of potential community responses to fragmentation was extensive and the second objective was restricted to functional indicators that have been well-studied and are likely to be influenced by different scales of fragmentation. Food webs are a reflection of complex ecosystem interactions (Vander Zanden et al. 2003). They provide a meaningful indication of ecosystem function (Thompson and Townsend 2005) and changes in hydrologic conditions are reflected in food web structure (Sternberg et al. 2008). Patterns of genetic structure are effective indicators of functional connectivity, or the ability of a species to disperse and re-populate areas (Tischendorf and Fahrig 2000), and genetic differentiation related to barriers can indicate reduced connectivity within a population (Manel and Holderegger 2013). These two functional indicators were chosen prior to conducting the literature review based on their ability to incorporate different spatial and temporal scales of fragmentation, thus providing different measures of fish response to fragmentation.

3.3 Methods

Structured Literature Review

A literature search was conducted in May 2017 with a time span from 1985 to 2016 using ISI Web of Science Core Collection to identify existing literature that could answer the question: what is the ecosystem response to river fragmentation? A string of multiple search terms intended to capture all literature related to physical fragmentation of rivers (e.g. fragmentation, regulation, dam, etc.) was used (Figure 1). Categories and research areas that were not applicable (e.g. medicine, education, engineering) were excluded, resulting in 28,059 search results. Titles were reviewed individually to remove any that were obviously irrelevant, followed by a more thorough abstract review. This screening resulted in 3,368 published studies related to physical fragmentation of rivers. These

results were further reduced to include only those assessing effects of fragmentation on fish using an Endnote search for the term 'fish' in any abstract, title, or keyword. Full texts for the resultant 1,199 published studies were then obtained (Figure 1).

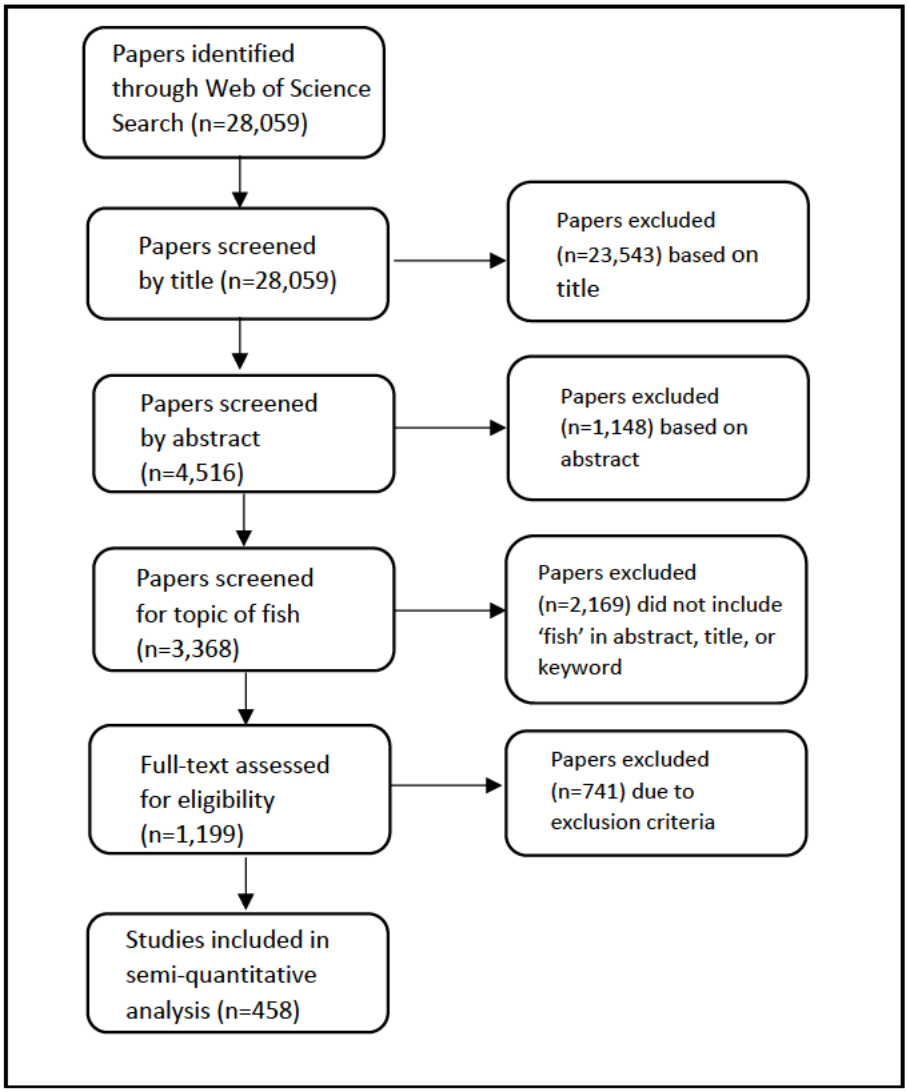

Figure 3-1. Flow of literature review process for Web of Science search for: 1985-2016; TOPIC: (River fragmentation) OR TOPIC: (river connectivity) OR TOPIC: (river disconnectivity) OR TOPIC: (dam) OR TOPIC: (dams) OR TOPIC: (weir) OR TOPIC: (weirs) OR TOPIC: ("flow regulation") OR TOPIC: ("flow alteration") OR TOPIC: (culvert) OR TOPIC: (culverts) OR TOPIC: ("water resource development") OR TOPIC: (refugia) OR TOPIC: ("expansion and contraction"). Conducted 14 May 2017; and 16 April 2018.

Full texts of the 1,199 publications were reviewed for relevancy based on a set of predetermined criteria. For the purposes of this literature review, only primary empirical research was of interest. Papers were excluded because they were not directly studying fish (e.g. subject was crayfish, fish habitat, or potential impact on fish) or because they fell under one of the following exclusion criteria:

- Narrative reviews
- Effects of restoration
- Evaluation of management response to fragmentation (e.g. fish passage and culvert design, or comparisons of dam operations)
- Studies within reservoir only without a river comparison
- Theoretical models
- Predicted impacts
- Effects during dam construction/closure only
- Response to connectivity without including fragmentation
- Response to flood after being fragmented

The final screening process resulted in 458 publications. The search process was expanded in April 2018 to include studies published in 2017, adding 37 publications. In total, this process identified 495 publications relevant to understanding fish community response to physical fragmentation of rivers.

Descriptive data from the 495 publications were then compiled into a database. Descriptive data included: 1) climate 2) discipline 3) location in river 4) time scale of study 5) dimension of fragmentation 6) type of fragmentation 7) agent of fragmentation 8) study design 9) subject of study 10) response variable and 11) community- or species-based study (Table 1). Categorization was based on information provided in the publication. If this descriptive information was not explicitly stated, a decision was made based on the other information provided.

Table 3-1. Categories of qualitative data extracted from studies that were identified in a systematic literature review on fish response to physical fragmentation of rivers.

Category	Level		Definition
Climate	• Equatorial • Arid • Warm	• Temperate • Snow • Polar	Köppen–Geiger Climate Classification (Kottek et al. 2006)
Discipline	• Biology • Hydrology • Geomorphology • Biogeochemical • Biology & Hydrology • Biology & Geomorphology	• Biology & Biogeochemical • Hydrology & Geomorphology • Hydrology & Biogeochemical • Mixed	The discipline in which the study was focused was determined based on tables, figures, and a review of the text to assess if the fish response by was explained by another field of study in addition to biology
Location in river	• Source • Upper • Middle • Lower	• Mouth • System • Mixed (various locations within multiple rivers)	Location of the study within the river (upper, middle, or lower) was assigned based on what was identified in the study text. If not explicitly identified, then an assignment was made based on the map provided or description of the location
Time scale	• Once • One Year	• Multi-year • Long Term	Once: data collected on one occasion One year: data collected multiple times in one year Multi-year: data collected more than one year, less than 10 Long term: data collected more than 10 years, includes genetic studies
Dimension of fragmentation	• Lateral • Longitudinal • Vertical • Temporal • Lateral & Longitudinal	• Lateral & Temporal • Longitudinal & Temporal • Mixed	

Type and Agent of fragmentation	• Natural
	  ○ Beaver
	  ○ Waterfall
	  ○ Natural Flow
	• Mixed
		• Anthropogenic
		  ○ Channelization Dam
		  ○ Flow Alteration
		  ○ Levee
		  ○ Weir
		  ○ Culvert	

Study Design	• BACI • Upstream vs Downstream • Regulated vs Unregulated • Modelling • Gradient • Over Time • Reservoir vs River • Other (radio-tagging)	Study design was assigned based on the primary level of comparison that was used to assess the fish response. Several studies incorporated multiple study designs (e.g. Upstream and downstream comparisons on both a regulated river and an unregulated river). For ease of summarizing, studies were assigned to the design in which the actual assessment of fish response to fragmentation was made. Gradient study design was comparisons made over a range of levels of fragmentation. Over time was assigned as the study design when the same sampling point was measured at different points in time, but no other comparison was made. Reservoir vs. river includes a measure of fish within a reservoir as well as within the lotic portion of the river. Studies that made a before and after comparison included at least one data point before habitat fragmentation occurred.
Response Variable	• Structural • Functional • Food web • Genetics • Community • Species-specific	Structural includes assemblage measures such as species composition, abundance, and diversity, as well as changes in habitat use or availability. Functional includes measures of productivity such as reproduction and growth rates. Dispersal metrics including recruitment, migration, and colonization. Also included changes in morphology. Studies including both structural and functional measures were categorized as the functional response variable.

To further explore the literature, the database was searched to identify studies that could address the specific questions of 1) does anthropogenic fragmentation alter food web dynamics? And 2) does anthropogenic fragmentation influence genetic differentiation in fish? Studies that were identified, based on descriptive data, as measuring a component of food web or genetic diversity were selected for further analysis. These topics were chosen as a focus prior to conducting the literature review based on an interest in assessing functional responses to fragmentation and the range of spatial and temporal scales these topics incorporate. The final selection process for the literature review to identify publications focused on fish likely excluded some relevant food web studies. To identify these potential studies, a keyword search of the 3,368 publications related to physical fragmentation of rivers was conducted. An additional 24 food web studies were identified and included in further analysis.

Eco Evidence

Further analysis was guided by the Eco Evidence framework, which provides a systematic approach to reviewing ecological literature (Nichols et al. 2011; Webb et al. 2012) and synthesizing multiple lines of evidence (Norris et al. 2012) to assess causality. The Eco Evidence framework can be used to establish if there is a causal link between an environmental stressor and an ecological response. Unlike traditional quantitative analysis methods, it allows for the inclusion of multiple types of studies and metrics. The framework utilizes a defined series of steps:

- formulating the problem and asking precise questions;
- reviewing the existing literature;
- weighting the evidence from the literature based on:
    - study design type,
    - number of reference sites, and
    - number of potentially impacted sites;
- using the evidence to reach a conclusion.

The Eco Evidence method guided the approach used here to establish the strength of evidence available to determine if there is a causal link between anthropogenic fragmentation in rivers and the ecological responses of both food web dynamics and genetic differentiation of fish.

An initial conceptual model for food web dynamics was designed with the intention of testing hypotheses that proposed specific components of flow regime (e.g. duration or frequency) and physical barriers (e.g. weir or levee) as drivers, and various measures of trophic interactions (e.g.

nutrient source or trophic position) as response variables. For example, testing the hypothesis that weirs change the predictability of flow events, altering food chain length. However, after an initial review of the literature it was apparent that the majority of relevant studies did not quantify or identify the specific change in the component of flow regime and few used consistent metrics to characterize food web structure or processes. Thus, based on what was available in the studies, our original hypotheses were revised to be tests of much broader relationships and were not confined to specific river types or geographic areas. The majority of studies could be grouped into two general categories: those assessing change in fish feeding due to the creation of reservoir, or lentic habitat; and those assessing change in trophic dynamics due to a dam altering flow. Based on this general distinction, we established two broad hypotheses: 1) river impoundment alters fish diet due to creation of lentic habitat, and 2) hydrological modification alters food web structure.

Table 3-2. Values for assigning weight to evidence based on study design. Based on Eco Evidence assessment method from Nichols et al. (2011).

Study Design	Weight
After impact only	1
Reference/control vs. impact (no before)	2
Before vs. after (no control)	2
Gradient response	3
BACI or modified BACI	4
Number of reference/control points	
0	0
1	2
>1	3
Number of impact points	
1	0
2	2
>2	3
Gradient response replicates	
<4	0
4	2
5	4
>5	6

Similar to the food web studies, many of the genetic studies did not quantify or identify a specific change in the flow regime. Again, based on what was available in the literature, a broad hypothesis was developed to include most of the available studies. The initial hypothesis was that physical barriers lead to genetic differentiation between populations above and below the barrier. This was then further refined based on the results of analysis. Permeability of the barrier was identified as an important factor influencing genetic differentiation (Bourne et al. 2011) and studies were separated into groups that indicated fish passage through the barrier was possible and those that indicated no passage was possible. Studies that did not indicate either possibility were discarded. The initial hypothesis was then tested on the two groups separately. Responses to fragmentation are also influenced by species-specific traits (Ewers and Didham 2006). For example, habitat specialists are at a higher risk of extinction due to habitat fragmentation than habitat generalist which are more adaptable (Ewers and Didham 2006). While assessing each trait and life-history strategy individually was beyond the scope of this study, there was enough evidence from studies on salmonids and studies on small-bodied species to test the initial hypothesis on these two groups separately. Although a range of traits that influence genetic response to fragmentation are incorporated within these two groupings, the prominence of these groups in the literature allowed the hypothesis to be tested on these more specific grouping.

The nature of the literature available did not allow for specific cause and effect type hypotheses to be tested, resulting in broad hypotheses that were not confined to specific groupings or river types. However, the Eco Evidence method for assessing the quality of the evidence available was still used as a way to objectively select literature. The study design and weight of evidence was assigned as described in Nichols et al. (2011). Briefly, studies are assigned weight based on study design and number of control and impact locations (Table 2). For example, a before-after-control-impact study design with more control and impact locations receives a higher weight than an after-only design with a low number of impact sites. Studies can be given a weight between 1 (low quality study) and 10 (high quality study). A threshold value of 20 is used to assign four possible outcomes: support for hypothesis, support for alternate hypothesis, inconsistent evidence, or insufficient evidence (Table 3). Weighting of studies was done using the default evidence weights.

Table 3-3. Decision criteria determined by total weight of evidence. Based on Eco Evidence assessment method from Nichols et al. (2011).

Total weight in support of hypothesis	**Total weight not in support of hypothesis**	**Conclusion**
≥20	<20	Support for hypothesis
≥20	≥20	Inconsistent evidence
<20	≥20	Support for alternative hypothesis
<20	<20	Insufficient evidence

3.4 Results

Literature Review

The 495 studies relevant to understanding fish community response to physical fragmentation of rivers were published in 113 different journals from 1988 to 2017. The number of studies published on the effect of habitat fragmentation on fish was fewer than 20 per year from 1988 through 2005. After 2005 the number of studies published increased dramatically, with a high of 46 published in 2016. The majority of the studies were not multidisciplinary, 61% focused only on the biological community. The studies that were multidisciplinary were mainly in the fields of biology and hydrology, followed by biology and geomorphology (Figure 2).

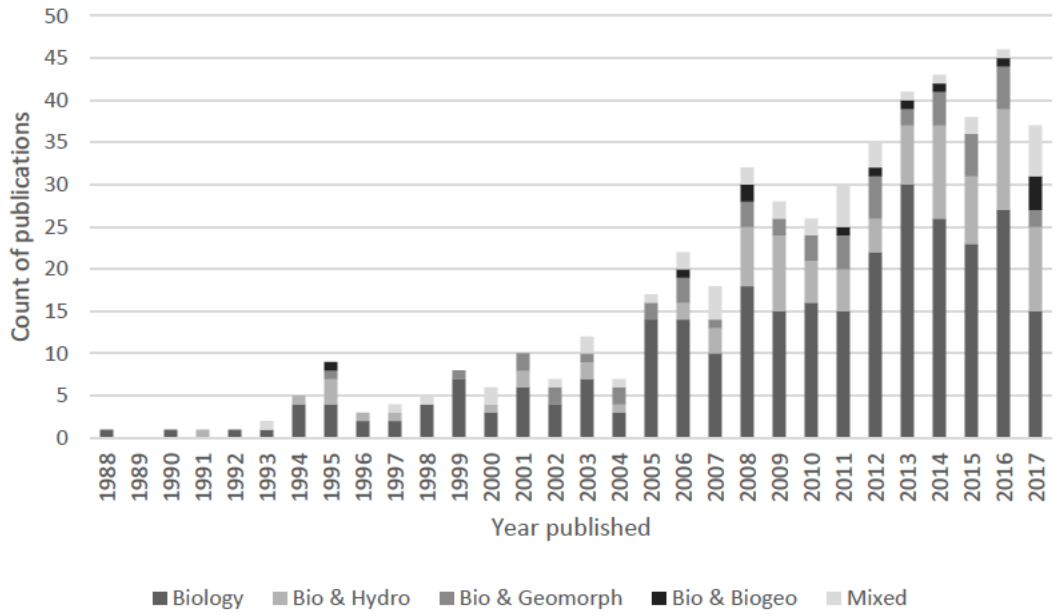

Figure 3-2. Year published and discipline of studies identified from a systematic literature review on the fish response to physical fragmentation of rivers

Climate and Location

Studies occurred primarily in temperate climates (67%), with half of the total studies occurring in warm temperate regions. Arid and semi-arid climates accounted for 19% of the studies and equatorial climates were 14% of the total. Studies have been conducted in all locations of the river. The largest percentage (28%) of studies were done in the upper river, followed by the middle (23%), and the lower (20%). The remaining studies (14%) covered the entire river.

Time Scale

Most studies (69%) used datasets spanning more than one year. Of these, long-term studies (those that collected data for more than 10 years) made up 30%, while multi-year studies (those that collected data over more than one to ten years) accounted for 39% of the total. Studies that collected data multiple times in one year made up 26% of the total and the remaining studies relied on fish community data that was collected on only one occasion (Figure 3).

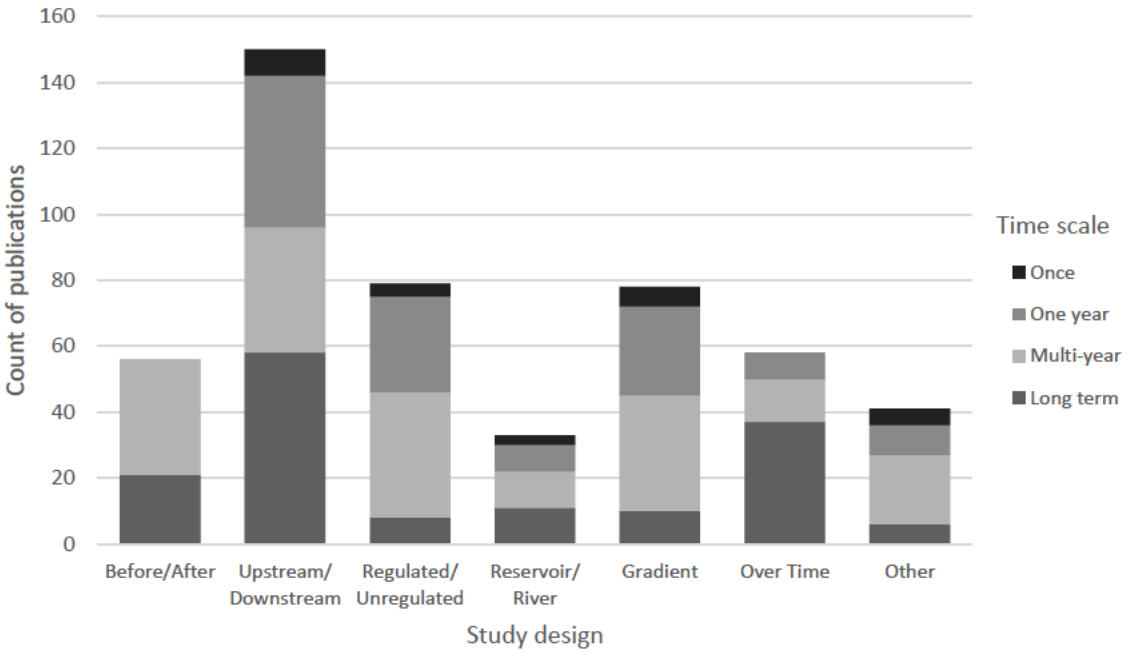

Figure 3-3. Time scale and design of studies identified in a systematic literature review on the fish response to physical fragmentation of rivers.

Type of Fragmentation

Overall, studies assessed multiple agents of fragmentation. Anthropogenic fragmentation was the most studied (81%) with dams being the most common agent (62%). Additional agents of anthropogenic fragmentation were, in declining order: weirs, channelization, culverts/road crossings, flow alteration, and levees. Natural fragmentation accounted for 14% of the studies, with the primary driver being the natural flow regime followed by beaver dams and waterfalls. The remaining 5% of studies assessed a mix of natural and anthropogenic causes of fragmentation (Table 4).

Table 3-4. Cause of fragmentation in studies identified in a systematic literature review on the fish response to physical fragmentation of rivers.

	Percent of total studies		Number of studies	Percent of total studies
Anthropogenic	81	Dam	305	61.6
		Weir	31	6 3
		Channelization	11	2.2
		Culvert	12	2.4
		Flow Alteration	18	3.6
		Levee	5	1.0
Both	5	Mixed	41	8 3
Natural	14	Natural Flow	51	10 3
		Beaver	14	2 8
		Waterfall	7	1.4
		Total	495	100.0

The vast majority of studies (70%) focused only on fragmentation of longitudinal connectivity. The primary cause of fragmentation of this dimension was dams (53%). Fragmentation of lateral connectivity was the focus of only 6% of the studies with the primary causes being dams and channelization. A further 10% of studies focused on fragmentation in a combination of lateral and temporal connectivity, driven primarily by the natural flow regime. The remaining studies assessed the fish response to different temporal scales of fragmentation (3%) or a combination of dimensions.

Study Design

The most commonly used study design was an upstream versus downstream design, accounting for 30% of the total. The regulated versus unregulated study design accounted for 16% of the total. A Before/After study design accounted for 11% of the total. The reservoir to river comparison design accounted for 7% of the total. An over-time design was used in 11% of the studies. Dams were the most common cause of fragmentation for these study designs. A gradient of fragmentation approach accounted for 16% of the studies. This study design was mainly used to assess a gradient of hydrological connectivity in unregulated rivers. The majority of studies in the 'other' category used telemetry to track fish movement and this category was 8% of the total (Figure 3).

Response Variable

A little over half of the studies focused on structural response variables (55%) assessing a measure of fish community assemblage (i.e. species composition, abundance, diversity). The remaining studies used functional measures of fishes. About a third of these were focused on genetic changes, 26% measured fish productivity, 21% assessed changes in dispersal, and 13% focused on food web attributes. Most studies (61%) included multiple species in their assessments (Figure 4).

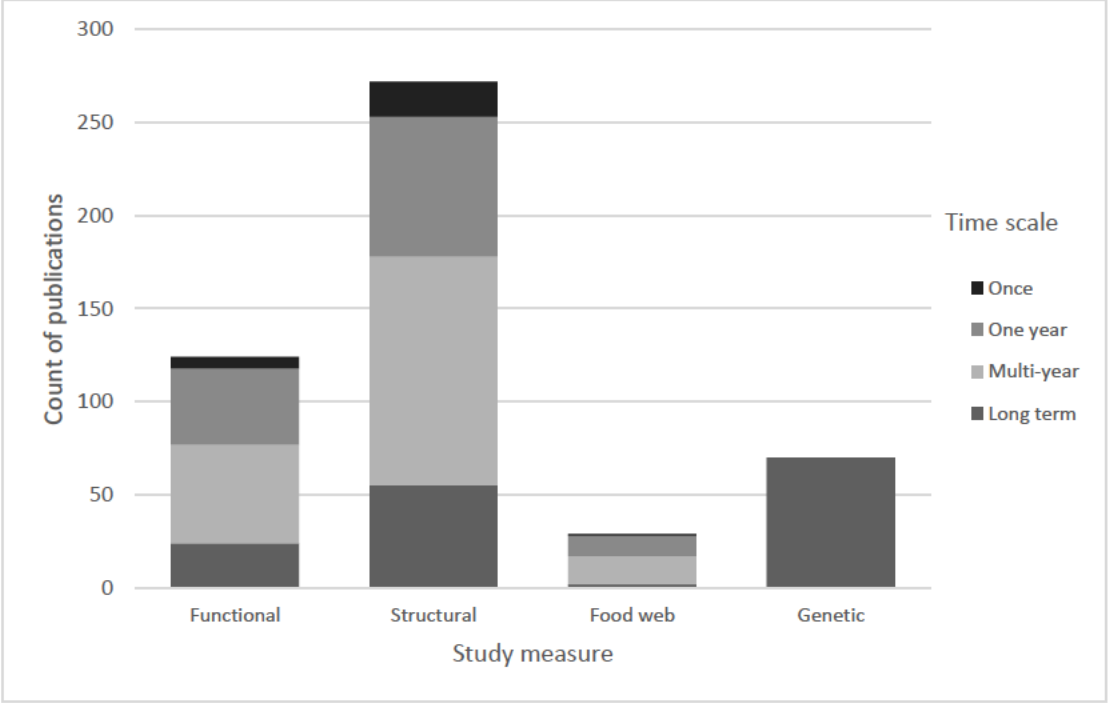

Figure 3-4. Time scale and response measure in studies identified in a systematic literature review on the fish community response to physical fragmentation of rivers.

Summary

In general, studies on the effects of physical fragmentation on fishes focused on dams as the cause of fragmentation along the longitudinal dimension. Most studies collected data for more than one year, but when genetic studies are removed from the long-term studies classification, only 81 studies, or 16%, consist of a dataset spanning more than 10 years. Only 21 studies, or 4%, used a long-term before and after study design. The majority of studies included more than one species, but most focused on changes in assemblage rather than functional community metrics.

Eco Evidence Analysis

Food Web Studies

Studies from the systematic literature review measuring the fish component of food webs and other trophic interactions or feeding behaviors were selected for use in the Eco Evidence analysis. Of the 53 studies identified from the literature review, only those in which the fish food web response or change in feeding behavior was assessed in relation to an identified anthropogenic cause were selected for further analysis. This resulted in 18 relevant studies. The remaining 35 studies were assessing natural flow variability, did not identify the anthropogenic cause, or were using a component of the food web but not studying the trophic interactions themselves (e.g. fatty acids as biomarkers). The majority of these studies identified a dam as the driver of change. One study examined the effects of a low-level weir (Baumgartner 2007) and was excluded from further analysis to make results more comparable.

The first cause-effect hypothesis tested was that river impoundment alters fish diet due to creation of lentic habitat. There were 13 studies containing evidence relevant to this hypothesis. Ten evidence items with a total weight of 58 supported this hypothesis and three evidence items with a total weight of 13 did not support this hypothesis (Table 5). The result is a conclusion of support for this hypothesis. There is evidence that the creation of lentic habitat alters fish diet.

The responses detected in the studies were mainly attributed to a change in resource availability. For example, several studies reported a shift from benthic-based food webs to an increased reliance on reservoir-derived pelagic food resources in downstream food webs (Delariva et al. 2013; Freedman et al. 2014; Mercado-Silva et al. 2009). Others found that feeding behavior of fish in both river and reservoir changed in response to the initial increase in small fish as a food resource (de Merona et al. 2001; Pereira et al. 2016). Some studies found seemingly contrasting patterns, for example Mazumder et al. (2016) and Kautza and Sullivan (2016) found a decreased range of food resources above a dam and within impounded reaches, while de Merona and Vigouroux (2006) found that fish within a reservoir exhibited increased omnivory. Finally, Penczak (1995) reported only a general but significant change in diet post-impoundment, and Alexandre et al. (2015) found that regulation resulted in fish maintaining a generalist diet year-round.

The studies that did not find a significant change in fish diet due to the creation of lentic habitat still identified a change in basal resources. Albrecht and Caramaschi (2003) found that an omnivorous fish was able to take advantage of increased autochthonous resources in a reservoir, but there was

no significant difference in feeding activity compared to the river. Similarly, Pereira et al. (2007) found a change in food resources but no significant spatial differences in isotope ratios. While there were spatial differences in isotope ratios between the river channel and reservoir, Kaymak et al. (2015) found that those differences were more closely linked to seasonal and landscape differences than the influence of a dam.

The second cause-effect hypothesis tested was that hydrological modification alters food web structure. Similar to the first hypothesis but assessing how hydrological modification influences different feeding guilds or trophic position rather than fish diet in general. There were seven studies relevant to this hypothesis. Four evidence items with a total weight of 24 did not support the hypothesis, and three evidence items with a total weight of 21 did support this hypothesis (Table 6). The result is a finding of inconsistent evidence that hydrological modification alters food web structure.

The primary hydrological modification was the stabilization of flows, resulting in habitat homogenization and stabilization of food resources. Studies that did not support this hypothesis identified that there was a change in nutrient resources or diet, but it had no significant influence on trophic position (Freedman et al. 2014; Mercado-Silva et al. 2009), food web length (Smokorowski et al. 2011), or niche breadth (Lik et al. 2017). Whereas studies that did support this hypothesis found that hydrological modification decreased trophic complexity (Cross et al. 2013; Turner et al. 2015), or had a variable but consistent change in stable isotope ratios (Delong and Thoms 2016).

Genetic Studies

Studies from the systematic literature review measuring fish genetic response were selected for use in the Eco Evidence analysis. Of these 70 studies, only those in which the fish genetic response to fragmentation was assessed in relation to an identified physical barrier were selected for further analysis. The majority of the remaining 35 studies identified a dam as the physical barrier, with four identifying a weir as the barrier.

The first hypothesis tested was that physical barriers will lead to genetic differentiation between populations above and below the barrier. There were 17 evidence items with a total weight of 132 that supported this hypothesis and 15 evidence items with a total weight of 110 that did not support this hypothesis. Three studies with a total weight of 26 had mixed results. The weight of the mixed evidence was included in both values (Table 7). The result was that there was inconsistent evidence on the link between physical barriers and genetic differentiation.

In studies that did support the hypothesis, the dam or weir acted as a barrier to fish movement, restricting gene flow among populations. Additional environmental factors associated with the barrier, such as water level variation (Ouellet-Cauchon et al. 2014), degraded habitat (Faulks et al. 2011) and presence of lentic habitat (Hudman and Gido 2013), also contributed to genetic differentiation. Although some studies had indications that the barrier may have influenced gene flow, tests for genetic differentiation were not significant (Dehais et al. 2010; Haponski et al. 2007; Tian et al. 2015) or could not be attributed to the anthropogenic barrier (Crookes and Shaw 2016). There were also cases where there was genetic differentiation, but it was more strongly correlated with historic processes or natural variation rather than the barrier (Clemento et al. 2009; Davis et al. 2015; McDougall et al. 2017; Underwood et al. 2016).

Three of the studies contained evidence that supported the hypothesis, as well as evidence that did not support the hypothesis. One of these studies assessed multiple species of fish and found that species' traits, such as size, determined if fragmentation influenced genetic structure (Blanchet et al. 2010). In addition to species' traits, the type of barrier also determined if there was support for the hypothesis. A barrier that only allowed fish movement during high flow years contributed to population subdivision only during low flow years, when fish passage was not possible (Peacock et al. 2016). A study assessing both high and low dams found that there was evidence in support of the hypothesis for the high dam, but not for the low dams (Zhao et al. 2016).

The initial hypothesis was reviewed due to the finding of inconsistent evidence. It was hypothesized that the presence of a fish bypass or otherwise selectively permeable fish passage can influence the effect of the barrier. Twenty-three of the 35 studies indicated whether fish passage was possible or not. The studies were separated into those with fish passage and those without and the original hypothesis was tested on each group separately.

There were 11 studies that identified some level of fish passage was possible, including the three studies that had mixed results in the initial hypothesis. The weight of these three studies was included as both supporting and not supporting the revised hypothesis. There were seven evidence items with a total weight of 48 in support of the hypothesis. There were seven evidence items with a total weight of 51 that did not support the hypothesis. Consequently, there was inconsistent evidence to make a conclusion about barriers causing genetic differentiation when fish passage is possible.

There were 12 studies that indicated no fish passage was possible. Seven evidence items with a weight of 36 supported the hypothesis and five evidence items with a weight of 27 did not support

the hypothesis. Again, there was inconsistent evidence to make a conclusion about barriers causing genetic differentiation when fish passage is not possible.

Within the nine studies on salmonids there was evidence in support of the hypothesis that physical barriers lead to genetic differentiation. There were three studies with a weight of 16 that did not support the hypothesis and six studies with a weight of 34 that did support the hypothesis. Within the smaller-bodied species, there was inconsistent evidence to make a conclusion. There were four studies with a weight of 22 that did not support the hypothesis, and four studies with a weight of 26 that did support the hypothesis.

3.5 Discussion

Literature Review

There is a large body of literature related to the ecological impacts of physical fragmentation of rivers, about 14% of which is focused on fishes. Despite increased research interest in recent years, there are still gaps in our understanding of the long-term impacts of anthropogenic fragmentation on fish. The majority of research has consisted of relatively short-term observational studies conducted after the impact has occurred, with no control reference condition. Dams were the most studied cause of fragmentation, with a resultant focus on longitudinal fragmentation. There was also a geographical bias in the distribution of studies with most studied rivers located in temperate climates. Similar to previous reviews on flow alteration, many studies did not address the topic holistically or fully quantify the relationship between the change and the ecological response (Lloyd et al. 2004; Poff and Zimmerman 2010). Understanding the mechanism for observed changes is challenging when studies do not quantify the change, and when the time scale of the study does not align with the response of interest (Souchon et al. 2008). The existing literature provides a wealth of information on observed ecological changes, but the gaps in the literature have important implications for how we can interpret the existing knowledge. The importance of these gaps was highlighted in the Eco Evidence analysis (Norris et al. 2012).

Eco Evidence

The initial intent of using the Eco Evidence method was to test specific hypotheses relating changes in components of the flow regime to a response in fishes, to better understand the scale at which fragmentation was affecting the biological community. A large number of studies did not quantify the change that occurred as a result of the anthropogenic fragmentation. As a result, specific hypotheses about drivers and response variables could not be tested. This highlighted the need for

more directed studies that quantify changes due to fragmentation in order to test mechanistic links. Although specific hypotheses could not be tested, the methodology of Eco Evidence was able to provide a systematic framework for collating a variety of evidence.

Food Webs

The food web studies identified in this literature review provided strong evidence that food webs are affected by impoundment via the creation of lentic habitat, and this impact extends to both upstream and downstream areas. A change in resource availability is an expected effect of impoundment. Reservoirs alter nutrient availability and productivity (Gunkel et al. 2003; Straškrábová et al. 2005); and influences the phytoplankton (Holz et al. 1997), zooplankton (Popp et al. 1996), benthic macroinvertebrate (Popp and Hoagland 1995), and fish (Albrecht et al. 2009; de Merona and Vigouroux 2006; Turgeon et al. 2016) communities. The reservoir aging process is well documented, and there can be a delayed response from many components of the ecosystem as the reservoir is in a transitional phase for many years (Gubiani et al. 2011; Petts 1987). 'Trophic upsurge' predicts that a reservoir will experience a period of trophic non-equilibrium before stabilizing in a trophic equilibrium phase (Kimmel and Groeger 1983; Turgeon et al. 2016). Processes assessed during this transitional phase may only reflect an intermediate stage and may not measure what the longer-term changes will be after the shift in selective pressures as the reservoir reaches some stable state. However, studies containing evidence in support of this hypothesis ranged in post-impoundment time from 18 months to more than 50 years and the evidence strongly indicated that there was a response in the food web to impoundment.

While there was evidence that the creation of lentic habitat influences food webs, there was inconsistent evidence that changing hydrology itself alters food web structure. The choice of metric used to assess the impact of altered hydrology likely influenced the result. The studies here all found that there was a change in basal resources for fishes' diet, but no change in metrics of food web structure such as trophic position and food web length. Dam impacted sites shifted toward pelagic based nutrients (Freedman et al. 2014; Mercado-Silva et al. 2009) and changed nitrogen baseline signatures (Smokorowski et al. 2011), but those changes didn't translate to altered trophic interactions among consumers. There was not enough evidence to support the hypothesis that changing hydrology alters food web structure, but the evidence did indicate that altered hydrology impacts other food web metrics.

Food webs themselves are complex systems influenced by a myriad of factors (Power and Dietrich 2002). Hydrological regime, river geomorphology, and water quality all influence food webs (Robson

et al. 2017). Nutrients supporting food webs are determined based on landscape features, which differ within a river basin and among river systems (Hoeinghaus et al. 2007). The bias in the literature towards rivers in temperate climates limits the knowledge we have on how various food webs function. Given the variability in landscapes and flow regimes of different river types, it is reasonable to expect a range of food web responses to fragmentation and regulation (Delong and Thoms 2016).

Genetic Differentiation

There was inconsistent evidence that physical barriers lead to genetic differentiation even when accounting for the possibility of fish passage. Gene flow is influenced by a number of landscape factors and life-history traits, complicating efforts to understand the impact of barriers (Davis et al. 2018). Many studies in this review did not clearly identify important factors such as the dispersal ability of the species and the permeability of the barrier. Of the 35 studies directly assessing the influence of a physical barrier, only 23 indicated if fish were able to move across the barrier. The three studies that had mixed results illustrate the influence of species traits and barrier permeability.

Physical barriers vary in their permeability (Fuller et al. 2015), which is often dynamic and also determined by species-specific traits (Bourne et al. 2011). Fishways themselves vary in effectiveness, and even if they mitigate the impact of barriers, they can still influence genetic differentiation (Gouskov et al. 2016). Two of the studies had mixed results due to the type and permeability of the barrier. The study by Zhao et al. (2016) found no differentiation across a low dam in a small non-migratory fish, but only unidirectional gene flow across a high dam. The study by Peacock et al. (2016) observed that for multiple species, movement across in-stream barriers was determined by river flow, only preventing movement and gene flow during periods of low water levels.

Species-specific traits such as dispersal ability and body size determine the impact of habitat fragmentation (Lean et al. 2017; Thomas 2000). Modelling of spatial genetic pattern suggests that dispersal ability of a species influences the time (i.e. number of generations) it takes to detect a barrier to movement, with low dispersal ability resulting in a long time lag before a genetic response is detected (Landguth et al. 2010). The study by Blanchet et al. (2010) supported this in response to weirs. The small-bodied, and thus lower dispersal ability, species was the least affected by fragmentation, and the intermediate size fish were most affected (Blanchet et al. 2010).

To identify if there was evidence to support whether species traits influence the genetic response to fragmentation, studies were further separated. The two general groupings with enough evidence to

test the hypothesis were salmonids and small-bodied species. Salmonids are typically large-bodied, capable of large-scale movements, and there is substantial variation within this family that likely influences genetic response to fragmentation (Fraser et al. 2011). Within the studies on salmonids there was evidence in support of the hypothesis that physical barriers lead to genetic differentiation. The smaller-bodied species were mostly represented by darters, a group that also contains a variety of traits including a range of movement potential (Haponski et al. 2007). Grouping the small-bodied species together found inconsistent evidence.

As demonstrated by multiple findings of inconsistent evidence that physical barriers lead to genetic differentiation, the genetic response to anthropogenic fragmentation is complex. The relatively large number of studies did allow testing the hypothesis on more specific groups, but of these groupings only the studies on salmonids had enough evidence in support of the hypothesis to make a conclusion. A recent meta-analysis by Schlaepfer et al. (2018) found that anthropogenic fragmentation of forests had a negative genetic impact on animals, but the strength of the response was determined by species-specific traits, permeability of unsuitable habitat, and the time since fragmentation. For rivers, studies on a wider range of species and traits are needed to build the evidence available to determine the genetic response to anthropogenic fragmentation.

Most cases of anthropogenic fragmentation have occurred relatively recently from an evolutionary perspective, and a time lag in genetic response should be expected (Keyghobadi 2007). The age of barriers in these studies, when indicated, ranged from 20 years to 800 years. The age of barrier did not determine if a study had evidence in support of the hypothesis. For a large population that is completely isolated it will take hundreds of generations to detect a genetic response to isolation (Gido et al. 2015). Additionally, in many of the studies there were multiple barriers of differing ages with varying degrees of permeability which also influences the time needed to detect a response. Species' response to habitat loss may be delayed when colonization potential exists only slightly below the threshold for persistence (Hanski 1998). Continuing long-term studies can help identify early signs of a response (Lindenmayer et al. 2012).

3.6 Conclusion

Disentangling the complex interactions of river ecosystems to understand the direct impact of anthropogenic fragmentation on fish is challenging. This systematic literature review revealed some important gaps in the research. The biased geographic distribution of studies, scarcity of BACI type study designs, and low number of studies on natural causes of fragmentation limits our baseline knowledge of fish responses to fragmentation. The attempt to test cause-effect relationships for

both food web and genetic responses to fragmentation further exposed some shortcomings of the existing research and highlighted specific aspects where more research is needed, which concurs with the literature review. There was strong evidence that the creation of lentic habitat alters food webs, and some evidence that species-specific traits influence genetic response. However, there was inconsistent evidence regarding the other general hypotheses. Many studies did not provide enough information to fully understand the driver of change or species' response, limiting the specific hypotheses that could be tested. Future research that quantifies the change in flow regime or habitat, and accounts for additional factors that influence species responses, will help us understand mechanistic links between fragmentation and ecological response.

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Table 3-5. Studies identified in a systematic literature review on the fish response to physical fragmentation of rivers which were included in Eco Evidence assessment.

Hypothesis 1: River impoundment alters fish diet due to creation of lentic habitat								
Reference	Title	Study Design Type	Control Independent Sampling Units	Impact Independent Sampling Units	Gradient Independent Sampling Units	Total Points	Supports hypothesis?	Age of Barrier
Delariva et al. (2013)	Diet and trophic structure of the fish fauna in a subtropical ecosystem: impoundment effects	Before vs. After (no control)	0	>2		5	Yes	18 months
de Merona and Vigouroux (2006)	Diet changes in fish species from a large reservoir in South America and their impact on the trophic structure of fish assemblages (Petit-Sant Dam, French Guiana)	After Impact Only	0	2		3	Yes	6 years
Pereira et al. (2016)	Effects of river damming in Neotropical piscivorous and omnivorous fish: feeding, body condition and abundances	BACI or MBACI	≥2	>2		9	Yes	15 months
Penczak (1995)	Food-Consumption by Fish Populations in the Warta River, Poland, before and after Impoundment	Before vs. After (no control)	0	2		4	Yes	4 years
Mazumder et al. (2016)	Impoundment constraint of fish niche diversity in a temperate Australian river	After Impact Only	0	2		3	Yes	>30 years
Freedman et al. (2014)	River of the dammed: longitudinal changes in fish assemblages in response to dams	Reference/Control vs. Impact	≥2	>2		8	Yes	>50 years
de Merona et al. (2001)	Short term effects of Tucurui Dam (Amazonia, Brazil) on the trophic organization of fish communities	Before vs. After (no control)	0	>2		5	Yes	3 years
Mercado-Silva et al. (2009)	The Effects of Impoundment and Non-Native Species on a River Food Web in Mexico's Central Plateau	After Impact Only	0	>2		4	Yes	15 years
Kautza and Sullivan (2016)	Anthropogenic and natural determinants of fish food-chain length in a midsize river system	Gradient Response Model			≥6	9	Yes	>50 years
Alexandre et al. (2015)	Food resources and cyprinid diet in permanent and temporary Mediterranean rivers with natural and regulated flow	Reference/Control vs. Impact	≥2	>2		8	Yes	>30 years
					Total Weight	**58**		
Albrecht and Caramaschi (2003)	Feeding ecology of *Leporinus friderici* (Teleostei; *Anostomidae*) in the upper Tocantins River, Central Brazil, before and after installation of a hydroelectric plant	Before vs. After (no control)	0	>2		5	No	1 year
Pereira et al. (2007)	Spatial variation in the stable isotopes of C-13 and N-15 and trophic position of *Leporinus friderici* (Characiformes, *Anostomidae*) in Corumba Reservoir, Brazil	After Impact Only	0	>2		4	No	4 years

(Kaymak et al. 2015)	Stable isotope analysis reveals relative influences of seasonal hydrologic variation and impoundment on assimilation of primary production sources by fish in the Upper Yesilırmak River, Turkey	After Impact Only	0	>2		4	No	>30 years
					Total Weight	**13**		

Table 3-6. Studies identified in a systematic literature review on the fish response to physical fragmentation of rivers which were included in Eco Evidence assessment.

Hypothesis 2: Hydrological modification alters food web structure								
Reference	Title	Study Design Type	Control Independent Sampling Units	Impact Independent Sampling Units	Gradient Independent Sampling Units	Total Points	Supports Hypothesis?	Age of Barrier
Delong and Thoms (2016)	Changes in the trophic status of fish feeding guilds in response to flow modification	Gradient Response Model			5	7	Yes	>40 years
Turner et al. (2015)	Retrospective stable isotope analysis reveals ecosystem responses to river regulation over the last century	Before vs. After (no control)	0	>2		5	Yes	>40 years
Cross et al. (2013)	Food-web dynamics in a large river discontinuum	Gradient Response Model			≥6	9	Yes	>30 years
					Total Weight	**21**		
Freedman et al. (2014)	River of the dammed: longitudinal changes in fish assemblages in response to dams	Reference/Control vs. Impact	≥2	>2		8	No	>50 years
Smokorowski et al. (2011)	Ecosystem level assessment of environmentally based flow restrictions for maintaining ecosystem integrity: a comparison of a modified peaking versus unaltered river	Reference/Control vs. Impact	≥2	>2		8	No	14 years
Lik et al. (2017)	Summer co-existence of small-sized cyprinid and percid individuals in natural and impounded stretches of a lowland river: food niche partitioning among fishes	Reference/Control vs. Impact	1	1		4	No	>20 years
Mercado-Silva et al. (2009)	The effects of impoundment and non-native species on a river food web in Mexico's Central Plateau	After Impact Only	0	>2		4	No	15 years
					Total Weight	**24**		

Table 3-7. Studies identified in a systematic literature review on the fish response to physical fragmentation of rivers which were included in Eco Evidence assessment.

Hypothesis 1: Physical barriers will lead to genetic differentiation between population above and below barrier

Reference	Title	Study Design Type	Control Independent Sampling Units	Impact Independent Sampling Units	Gradient Independent Sampling Units	Total Points	Supports Hypothesis?	Fish Passage?	Age of oldest Barrier (years)	Species Trait
Peacock et al. (2016)	Native fishes in the Truckee River: Are in-stream structures and patterns of population genetic structure related?	Gradient Response Model			≥6	9	Mixed	Flow Dependent	>70	
Zhao et al. (2016)	Effects of dam structures on genetic diversity of freshwater fish *Sinibrarna macrops* in Min River, China	Gradient Response Model			≥6	9	Mixed	Mixed		
Blanchet et al. (2010)	Species-specific responses to landscape fragmentation: implications for management strategies	Reference/Control vs. Impact	≥2	>2		8	Mixed	Mixed	>70	Small-Bodied (and Large-Bodied)
					Total Weight	**26**				
Heggenes and Roed (2006)	Do dams increase genetic diversity in brown trout (*Salmo trutta*)? Microgeographic differentiation in a fragmented river	Gradient Response Model			≥6	9	Yes	No	>90 - 50	Salmonid
(Hanfling and Weetman 2006)	Concordant genetic estimators of migration reveal anthropogenically enhanced source-sink population structure in the river sculpin, *Cottus gobio*	Gradient Response Model			≥6	9	Yes	Unknown	200	Small-Bodied
(Hansen et al. 2014)	The effects of Medieval dams on genetic divergence and demographic history in brown trout populations	After Impact Only	0	>2		4	Yes	No	800	Salmonid
(Beneteau et al. 2009)	The effects of river barriers and range expansion of the population genetic structure and stability in greenside	After Impact Only	0	>2		4	Yes	Yes		Small-Bodied

	darter (*Etheostoma blennioides*) populations									
(Fluker et al. 2014)	The effects of riverine impoundment on genetic structure and gene flow in two stream fishes in the Mobile River basin	Reference/Control vs. Impact	≥2	>2		8	Yes	Unknown		Small-bodied
Hudman and Gido (2013)	Multi-scale effects of impoundments on genetic structure of creek chub (*Semotilus atromaculatus*) in the Kansas River basin	Reference/Control vs. Impact	1	>2		7	Yes	Unknown		
Ardren and Bernall (2017)	Dams impact westslope cutthroat trout metapopulation structure and hybridization dynamics	After Impact Only	0	>2		4	Yes	Flow Dependent	>60	Salmonid
Faulks et al. (2011)	The role of anthropogenic vs. natural in-stream structures in determining connectivity and genetic diversity in an endangered freshwater fish, Macquarie perch (*Macquaria australasica*)	After Impact Only	0	>2		4	Yes	No		
Ouellet-Cauchon et al. (2014)	Landscape variability explains spatial pattern of population structure of northern pike (*Esox lucius*) in a large fluvial system	After Impact Only	0	>2		4	Yes	Unknown	>50	
Bessert and Orti (2008)	Genetic effects of habitat fragmentation on blue sucker populations in the upper Missouri River (*Cycleptus elongatus Lesueur*, 1918)	Reference/Control vs. Impact	≥2	>2		8	Yes	No	>60	
Yamamoto et al. (2004)	Genetic differentiation of white-spotted charr (*Salvelinus leucomaenis*) populations after habitat fragmentation: Spatial-temporal changes in gene frequencies	After Impact Only	0	>2		4	Yes	No	>30	Salmonid
Gouskov et al. (2016)	Fish population genetic structure shaped by hydroelectric power plants in the upper Rhine catchment	Gradient Response Model			≥6	9	Yes	Yes	>80	
Samarasin et al. (2017)	After 100 years: hydroelectric dam-induced life-history divergence and	Reference/Control vs. Impact	≥2	>2		8	Yes	No	>70	Salmonid

	population genetic changes in sockeye salmon (*Oncorhynchus nerka*)									
Pamponet et al. (2008)	A multi-approach analysis of the genetic diversity in populations of *Astyanax aff. bimaculatus* Linnaeus, 1758 (Teleostei: Characidae) from Northeastern Brazil	Reference/Control vs. Impact	1	2		6	Yes	Unknown	40	
Roberts et al. (2013)	Distance, dams and drift: what structures populations of an endangered, benthic stream fish?	After Impact Only	0	>2		4	Yes	No	>90 - 50	Small-Bodied
Meldgaard et al. (2003)	Fragmentation by weirs in a riverine system: A study of genetic variation in time and space among populations of European grayling (*Thymallus thymallus*) in a Danish river system	Before vs. After (no control)		>2		5	Yes	Yes	>50	Salmonid
Raeymaekers et al. (2008)	Modeling genetic connectivity in sticklebacks as a guideline for river restoration	Gradient Response Model			≥6	9	Yes	Unknown	>70	Small-Bodied
					Total Weight	**106**				
Haponski et al. (2007)	Genetic divergence across a low-head dam: A preliminary analysis using logperch and greenside darters	Reference/Control vs. Impact	1	2		6	No	No	>70	Small-Bodied
Davis et al. (2015)	The influence of historical and contemporary landscape variables on the spatial genetic structure of the rainbow darter (*Etheostoma caeruleum*) in tributaries of the upper Mississippi River	After Impact Only	0	>2		4	No	Unknown		Small-Bodied
Reid et al. (2008)	Population structure and genetic diversity of black redhorse (*Moxostoma duquesnei*) in a highly fragmented watershed	Gradient Response Model			≥6	9	No	Mixed		
Tian et al. (2015)	Population genetic structure of *Siniperca chuatsi* in the middle reach of the Yangtze River inferred from mitochondrial DNA and microsatellite loci	After Impact Only	0	>2		4	No	Unknown	>50	

Skalski et al. (2008)	Genetic structure of creek chub, a headwater minnow, in an impounded river system	Reference/Control vs. Impact	≥2	>2	8	No	Unknown	>70 - 30	
Heggenes et al. (2006)	Genetic structure in relation to movements in wild European grayling (*Thymallus thymallus*) in three Norwegian rivers	Reference/Control vs. Impact	≥2	>2	8	No	Yes	>60 - 20	Salmonid
Ferreira et al. (2017)	Genetic structure and diversity of migratory freshwater fish in a fragmented Neotropical river system	After Impact Only	0	>2	4	No	Yes	>30	
McDougall et al. (2017)	Rethinking the influence of hydroelectric development on gene flow in a long-lived fish, the Lake Sturgeon *Acipenser fulvescens*	After Impact Only	0	>2	4	No	No	>70	
Garcez et al. (2011)	Population structure of the migratory fish *Prochilodus lineatus* (*Characiformes*) from rio Grande basin (Brazil), an area fragmented by dams	After Impact Only	0	>2	4	No	Yes		
Olsen et al. (2016)	Genetic diversity and divergence in the fountain darter (*Etheostoma fonticola*): implications for conservation of an endangered species	After Impact Only	0	>2	4	No	Unknown	>50	Small-Bodied
Crookes and Shaw (2016)	Isolation by distance and non-identical patterns of gene flow within two river populations of the freshwater fish *Rutilus rutilus* (L. 1758)	Reference/Control vs. Impact	≥2	>2	8	No	Unknown	>100	
Clemento et al. (2009)	Population genetic structure and ancestry of *Oncorhynchus mykiss* populations above and below dams in south-central California	After Impact Only	0	>2	4	No	No	>80 - 40	Salmonid
(DeHaan et al. 2011)	Genetic population structure of Olympic Peninsula bull trout populations and implications for Elwha dam removal	After Impact Only	0	>2	4	No	No	>80	Salmonid
(Underwood et al. 2016)	Population connectivity and genetic structure of burbot (*Lota lota*)	After Impact Only	0	>2	4	No	No	>80	

	populations in the Wind River Basin, Wyoming					
Dehais et al. (2010)	Microgeographic genetic isolation in chub (*Cyprinidae: Squalius cephalus*) population of the Durance River: estimating fragmentation by dams	Gradient Response Model	≥6	9	No	Unknown
			Total Weight	**84**		

Higher Degree Research Thesis by Publication
University of New England

STATEMENT OF ORIGINALITY

(To appear at the end of each thesis chapter submitted as an article/paper)

We, the Research Master/PhD candidate and the candidate's Principal Supervisor, certify that the following text, figures and diagrams are the candidate's original work.

Type of work	Page number/s
Journal article and all-encompassing work	

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STATEMENT OF AUTHORS' CONTRIBUTION

(To appear at the end of each thesis chapter submitted as an article/paper)

We, the Research Master/PhD candidate and the candidate's Principal Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated in the *Statement of Originality*.

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Fish fins as a non-lethal alternative to muscle tissue in stable isotope studies of food webs in an Australian river

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4.1 Abstract

This study found a strong relationship between fin and muscle tissue in three Australian fish species, showing that non-lethally collected fin tissue can be used as a proxy for muscle tissue in isotopic trophic studies. We hypothesised that a strong linear relationship exists between fin and muscle $\delta^{13}C$ and $\delta^{15}N$ isotope ratios, and conversion equations can be developed where differences exist. We analysed fin and muscle tissues of three common species from the Barwon-Darling River, New South Wales, Australia. There were significant differences between fin and muscle $\delta^{13}C$ values for all species, and fin tissue was a good predictor of muscle tissue $\delta^{13}C$ (r^2=0.77 for all species). The relationship between $\delta^{15}N$ values was less consistent, with a significant difference found in one species, fin tissue was still a good predictor (r^2=0.72 for all species). Developing species-specific tissue conversion models result in the least amount of error, but regional models result in similar error and are more accurate than general global models. These results are consistent with prior studies of different species. This study provides tissue conversion models for three species common to Australian lowland rivers, facilitating the inclusion of fish in food web studies with non-lethal collection methods.

4.2 Introduction

The use of stable isotopes to study trophic dynamics has been well established (Fry 2006; Layman *et al.* 2007; Post 2002). Carbon stable isotope ratios ($^{13}C/^{12}C$) reflect sources of food consumed; while nitrogen stable isotope ratios ($^{15}N/^{14}N$) indicate a consumer's trophic position (Peterson and Fry 1987). Isotopic analysis has been widely used in studies of trophic ecology of fish (Boecklen *et al.* 2011) and has been shown to be a reliable method of measuring energy flow (Deniro and Epstein 1981; Finlay *et al.* 2002), characterising trophic niche (Bearhop *et al.* 2004), estimating food chain length (Hoeinghaus *et al.* 2008), as well as reconstructing historical food webs (Turner *et al.* 2015; Vander Zanden *et al.* 2003).

In studies of vertebrates, muscle tissue is frequently chosen for stable isotope analysis (Boecklen *et al.* 2011). In fish, white muscle tissue is commonly used as it reflects isotopic composition of diet with minimal within tissue variability (Pinnegar and Polunin 1999). However, standard methods for collection of muscle tissue requires the animal to be sacrificed (Boecklen *et al.* 2011). The use of biopsy plugs to collect samples of muscle tissue from live fish has been used successfully, with no indication of increased fish mortality (Hanisch *et al.* 2010; Henderson *et al.* 2016). Still, this is an invasive option and may not be appropriate for use on threatened species and systems, vulnerable

populations, or small-bodied or juvenile fish. Fishes often represent higher trophic positions and their inclusion in studies provides vital information on food web function. However, the larger size of some species limits their abundance (Trebilco *et al.* 2013), and the removal of large consumers may result in broader-scale impacts to the ecosystem (Estes *et al.* 2011). An alternative to muscle tissue will enable the inclusion of fishes in food web studies with minimal impact to the wider community and avoid the ethical implications of invasive sample collection.

Recent studies have explored non-lethal and less invasive alternatives as a substitute for fish muscle tissue, such as mucous and scales (Busst and Britton 2017; Church *et al.* 2009; Nolan and Britton 2018; Vašek *et al.* 2017; Winter *et al.* 2019). Most promising has been the use of fin tissue. The use of caudal fin clips is non-lethal and minimally invasive. Fin tissue is able to regenerate (German and Miles 2010; Wills *et al.* 2008), and clipping likely has minimal impact on behaviour (Champagne *et al.* 2008; Dietrich and Cunjak 2006). Several studies have indicated that fin tissue provides comparable results to muscle tissues for both $\delta^{13}C$ and $\delta^{15}N$ (Busst *et al.* 2015; Jardine *et al.* 2011; Kelly *et al.* 2006; Sanderson *et al.* 2009; Tronquart *et al.* 2012).

Experimental study has shown that fin and muscle tissue integrate comparable time periods and have similar isotopic turnover rates (Suzuki *et al.* 2005), though this relationship can vary slightly by species and growth rate (Busst and Britton 2017). Studies comparing muscle and fin tissue have found that although the tissues have different elemental composition, the relationship is consistent and predictable (Busst and Britton 2016; Hanisch *et al.* 2010; Sanderson *et al.* 2009). This relationship suggests that fin tissues can be used directly for trophic studies (Hanisch *et al.* 2010; Jardine *et al.* 2005). However, differences between tissues have the potential to influence interpretation of isotope results (Hayden *et al.* 2017), and the relationship between tissues should be accounted for. Applying a correction factor makes fin values more directly comparable to muscle tissue (Sanderson *et al.* 2009). General tissue conversion models reduce error, but species-specific models result in a higher degree of accuracy (Busst *et al.* 2015; Tronquart *et al.* 2012) due to factors such as climate, diet, and growth rates which influence isotopic composition and tissue turnover rates (Martínez del Rio *et al.* 2009). Establishing the relationship between the isotopic composition of muscle and fin tissue, and developing species-specific conversion factors are necessary to use fins as a non-lethal method for stable isotope analysis of fish tissues.

The aim of this study was to determine if caudal fin tissue can be used as a proxy for dorsal muscle tissue in studies of trophic ecology of three fish species common and ecologically important in Australian lowland rivers: common carp *Cyprinus carpio,* a non-native, invasive species; golden perch

Macquaria ambigua, a native opportunistic carnivore; and bony bream *Nematalosa erebi,* a widespread native detritivore.

On the basis of existing research on temperate and tropical species (Jardine *et al.* 2011; Tronquart *et al.* 2012), we hypothesised that there will be a strong, consistent linear relationship between fin and muscle $\delta^{13}C$ and $\delta^{15}N$ isotope ratios. Where differences between the tissues exists, a linear model can be used to convert fin isotope values to equivalent muscle values. We compared the $\delta^{13}C$ and $\delta^{15}N$ values of non-lethally collected dorsal muscle and caudal fin tissue to establish if there is a consistent relationship between the two tissue types. Linear models were developed for these three species to use fin tissue as a proxy in food web studies that include, or are to be compared to, studies using muscle tissue.

4.3 Materials and methods

Study area

The Barwon-Darling River in New South Wales, Australia is a low gradient river with a semi-arid climate. It has a catchment area of 650,000 km^2, 60% of which is less than 300 m above sea level, and has a main channel length of 3,100 km (Boys and Thoms 2006). This river has one of the most variable flow regimes in the world (Puckridge *et al.* 1998), with flows exhibiting seasonality, existing for most of the time in a low flow stage (Thoms *et al.* 2004). Sampling was conducted during a drought when the river experienced several extended cease-to-flow periods. There were no connecting flows during the sampling period (WaterNSW 2020). Samples were collected from 12 sites along a 1,390 km stretch of river between Mungindi and Menindee, NSW (Fig. 1). Samples used in this study were collected opportunistically during sample collection for a larger study. Fish were sampled using standard boat and backpack electrofishing methods (MDBC 2008) in September 2018 and March 2019.

Sample Collection

Fin clips and biopsy plugs used in this study were taken from 37 fish, including from 11 *C. carpio* that ranged in fork length between 283 and 670 mm, 15 *M. ambigua* with length between 280 and 438 mm, and 11 *N. erebi* with length between 195 and 325 mm (Table 1). Samples were taken from live fish which were released at the site of collection, with the exception of some *C. carpio* individuals which were humanely euthanised. Biopsy plugs of fish muscle tissue were collected following a protocol adapted from Baker *et al.* (2004) and Henderson *et al.* (2016). Tissue plugs were collected

below and towards the posterior end of the dorsal fin with a 5 mm disposable biopsy punch. Scales were removed from the procedure area and the biopsy punch was inserted to a depth of 5 mm. The

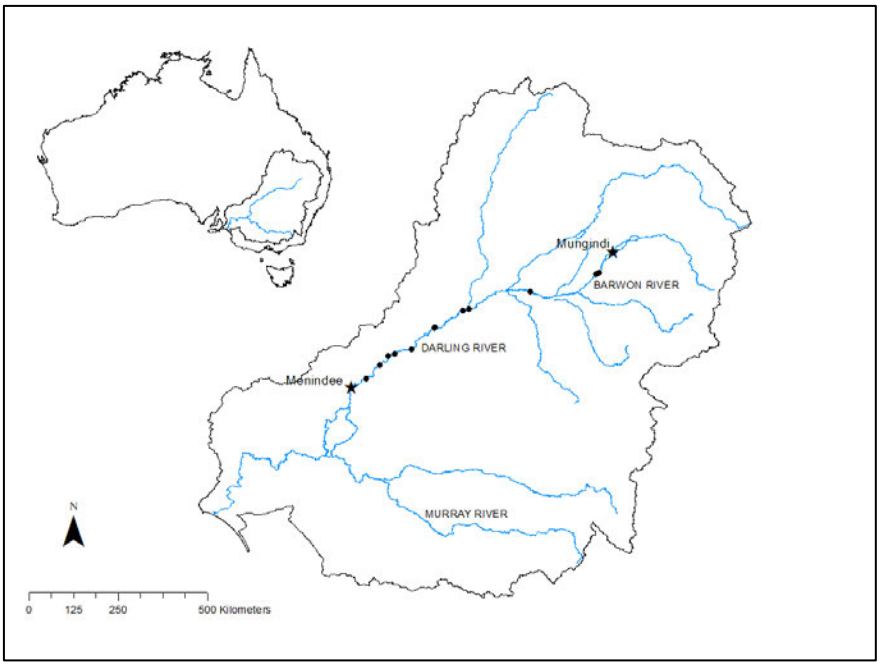

Figure 4-1. Locations of sample collection sites within the Murray-Darling Basin, New South Wales, Australia.

wound was filled with a methylcellulose based powder-gel in an attempt to reduce potential infection. Skin was removed and the tissue plug rinsed with distilled water. All samples were kept cool, and frozen within 24 hours of collection.

Fin tissue was taken from the distal portion of the upper caudal fin. Location of sample collection was standardized to collect from the tip of the caudal fin following suggestions of Hayden *et al.* (2015). At least 1 cm^2 of tissue including both membrane and ray was collected from small individuals, with more tissue collected from larger fish. Samples were kept cool, rinsed with distilled water, and frozen within 24 hours of collection.

Stable isotope preparation method

In the laboratory, muscle and fin tissue were dried at 60°C for 48 hours. Tissues were ground to a fine powder and weighed into tin capsules. Carbon and nitrogen were analysed separately with 2 ± 0.05 mg of powder for carbon, and 0.4 ± 0.05 mg for nitrogen. Samples were combusted in a continuous flow isotope ratio mass spectrometer (Sercon 20-22, Sercon Limited, Cheshire, U.K.) at University of New England, Armidale, Australia. Each run included a laboratory standard every 20 samples to account for equipment drift and estimate error. Analytical precision was estimated to be

0.3‰ for $\delta^{13}C$ and 0.6‰ for $\delta^{15}N$. Lipid content for both muscle and fin tissue was variable with C:N ratio of some samples >4, all $\delta^{13}C$ values were corrected for lipids following Post *et al.* (2007).

Data analysis

The elemental composition of muscle and fin tissue was compared with paired samples t-tests between all %C and %N pairs. To determine if a conversion factor is necessary, paired samples t-tests were used to determine if $\delta^{13}C$ values and $\delta^{15}N$ values were significantly different between tissue types. All data were normally distributed (Shapiro-Wilk normality test). Simple linear regression of fin tissue with muscle tissue $\delta^{13}C$ and $\delta^{15}N$ values, for all fish combined and for each species individually, was used to examine the relationship between tissues. Linear regression was also used to examine if there was a relationship between isotope values and fish fork length or year collected. Date was included as a factor to test if worsening drought conditions between sampling episodes influenced results. Models to convert the isotopic signatures of fin tissue to those of muscle tissue were generated using linear regression analysis. One sample of *N. erebi* muscle did not have results for isotopic analysis of nitrogen and was only included for analysis of carbon (Table 1).

The robustness of the models for estimating muscle isotope values was tested by comparing the mean residuals from each model, similar to methods by Busst *et al.* (2015) and Tronquart *et al.* (2012). Models generated from linear regression were used to convert $\delta^{13}C$ and $\delta^{15}N$ values of fin tissue to the equivalent muscle tissue values. Residuals of the species-specific models developed here were compared with the all-species model and general models developed by Tronquart *et al.* (2012) (hereafter referred to as "Tronquart model") and Jardine *et al.* (2011) (hereafter referred to as "Jardine model"), as well as the species-specific model for *Nematalosa sp.* developed by Jardine *et al.* (2011). Due to small sample sizes, data used to develop the models were also used to test the models. Welch two sample t-tests were used to determine if absolute residuals from each model were significantly different from the species-specific models developed here. Paired samples t-tests were used to determine if fin isotope values converted with models were different than observed muscle isotope values. All tests and regression analyses were done with R software V3.6.1 (R Core Team 2018).

Method considerations

To aid the design of future studies, we determined the minimum size of fin clip needed to provide enough tissue for stable isotope analysis. We measured the surface area of a subset fin clips and the resulting dry weight it provided. These measurements were made using a different set of fin samples consisting of different species collected from the same sample sites (unpublished data).

Table 4-1. Mean (± s.d.) fork length and elemental composition (%C, %N, and C:N ratio) of fin tissue and muscle tissue for fish species collected from an Australian dryland river.

Species name	Fork length (mm)	*n*	Collection date	%C in muscle	%C in fin	%N in muscle	%N in fin	C:N in muscle	C:N in fin
Cyprinus carpio	438.9 ± 123.6	11	Mar 2019	42.3 ± 4.2	25.5 ± 4.4	12.0 ± 1.4	7.5 ± 1.0	3.5 ± 0.3	3.4 ± 0.3
Macquaria ambigua	342.0 ± 44.3	15	Sept 2018; Mar 2019	44.2 ± 3.8	28.9 ± 2.8	11.2 ± 1.3	8.4 ± 1.1	4.0 ± 0.6	3.5 ± 0.4
Nematalosa erebi	273.7 ± 39.9	11 (C) 10 (N)	Sept 2018; Mar 2019	40.6 ± 3.7	20.9 ± 2.0	12.6 ± 1.7	7.0 ± 1.1	3.2 ± 0.4	3.0 ± 0.6
All fish	352.6 ± 98.6	37		42.6 ± 4.1	25.5 ± 4.6	11.8 ± 1.5 (n=36)	7.7 ± 1.2	3.6 ± 0.6 (n=36)	3.3 ± 0.5

4.4 Results

Stable isotope analysis

The C:N ratio was similar between fin (3.3 ± 0.5) and muscle (3.6 ± 0.6) tissue (Table 1), but the two tissues differed in their elemental composition. Comparisons between %C of muscle and fin tissue and %N of tissues were significant for all fish combined (%C: $t_{36} = -22.12$, $P < 0.001$; %N: $t_{35} = -11.84$, $P < 0.001$) and all species individually ($P < 0.001$). Fin tissue was consistently lower in %C (fin = 25.5 ± 4.6%; muscle = 42.6 ± 4.1%) and %N (fin = 7.7 ± 1.2%; muscle = 11.8 ± 1.5%) than muscle tissue (Table 1).

Fin tissue was enriched in ^{13}C relative to muscle tissue for all species. The difference between fin and muscle $\delta^{13}C$ values was significant for all fish combined ($t_{36} = 10.18$, $P < 0.001$) as well as each species separately (*C. carpio*: $t_{10} = 5.49$, $P < 0.001$; *M. ambigua*: $t_{14} = 6.51$, $P < 0.001$; *N. erebi*: $t_{10} = 5.24$, $P < 0.001$) (Table 2). $\delta^{15}N$ values were more similar between tissues, but the relationship varied by species. There was no significant difference when all species were combined ($t_{35} = -1.61$, $P = 0.117$), for *C. carpio* ($t_{10} = -1.06$, $P = 0.314$), or for *M. ambigua* ($t_{14} = 1.10$, $P = 0.289$), but the difference between $\delta^{15}N$ values of tissues in *N. erebi* was significant ($t_9 = -4.23$, $P = 0.002$) (Table 2). The lack of significant differences between $\delta^{15}N$ fin and muscle values for both *C. carpio* and *M. ambigua* indicated that a tissue conversion factor is not necessary. However, for consistency fin values for these species were converted to equivalent muscle values in further analyses.

Linear regression

There was a strong linear relationship between muscle and fin $\delta^{13}C$ values for all fish combined ($r^2 = 0.77$, $P < 0.001$) and significant relationships for each species ($P < 0.01$) (Table 2, Fig. 2). There were also significant linear relationships between muscle and fin $\delta^{15}N$ values for all fish combined ($r^2 = 0.72$, $P < 0.001$) and significant relationships for each species ($P < 0.05$) (Table 2, Fig. 2). There was no significant relationship between tissue isotope values and fish length or date collected. The model for *M. ambigua* accounted for the least amount of variation of three species for both $\delta^{13}C$ and $\delta^{15}N$, while the model for *N. erebi* accounted for the most variation (Table 2). Slopes of regression lines were less than 1.0 for all relationships with the exception of $\delta^{13}C$ in *N. erebi*.

Table 4-2. Linear regression equations for $\delta^{13}C$ and $\delta^{15}N$ in fin tissue *vs*. muscle tissue with 95% confidence intervals (CI) of the regression slope and mean (± s.d.) isotope value difference (*indicates difference is significant) for three fish species from an Australian dryland river. Additional published regression equations from Jardine *et al.* (2011) and Tronquart *et al.* (2012).

Species name	Regression equation	r^2	p-value	Slope 95% CI	Mean (fin-muscle) (‰)
Cyprinus carpio	Muscle $\delta^{13}C = 0.7360$ x fin $\delta^{13}C - 8.0028$	0.74	<0.001	0.4069 - 1.0651	1.63 ± 0.99*
Macquaria ambigua	Muscle $\delta^{13}C = 0.5517$ x fin $\delta^{13}C - 12.4129$	0.42	0.009	0.1657 - 0.9378	1.53 ± 0.91*
Nematalosa erebi	Muscle $\delta^{13}C = 1.3086$ x fin $\delta^{13}C + 6.9075$	0.84	<0.001	0.8756 - 1.7417	1.32 ± 0.84*
All fish	Muscle $\delta^{13}C = 0.8472$ x fin $\delta^{13}C - 5.3096$	0.77	<0.001	0.6866 - 1.0078	1.50 ± 0.90*
Jardine *Nematalosa*	Muscle $\delta^{13}C = 0.98$ x fin $\delta^{13}C - 0.93$	0.92	<0.001		0.4 ± 1.3
Jardine model	Muscle $\delta^{13}C = 0.89$ x fin $\delta^{13}C - 3.27$	0.91	<0.001		0.9 ± 1.0
Tronquart model	Muscle $\delta^{13}C = 0.82$ x fin $\delta^{13}C - 5.89$	0.85	<0.001		
Cyprinus carpio	Muscle $\delta^{15}N = 0.3869$ x fin $\delta^{15}N + 8.0170$	0.46	0.02	0.0693 - 0.7045	-0.43 ± 1.33
Macquaria ambigua	Muscle $\delta^{15}N = 0.4671$ x fin $\delta^{15}N + 7.1422$	0.38	0.02	0.1062 - 0.8279	0.30 ± 1.05
Nematalosa erebi	Muscle $\delta^{15}N = 0.7904$ x fin $\delta^{15}N + 3.3677$	0.84	<0.001	0.5089 - 1.0720	-1.17 ± 0.88*
All fish	Muscle $\delta^{15}N = 0.5794$ x fin $\delta^{15}N + 5.5924$	0.72	<0.001	0.4524 - 0.7063	-0.33 ± 1.23
Jardine *Nematalosa*	Muscle $\delta^{15}N = 1.06$ x fin $\delta^{15}N - 0.29$	0.82	<0.001		-0.2 ± 0.8
Jardine model	Muscle $\delta^{15}N = 0.81$ x fin $\delta^{15}N + 1.73$	0.56	0.008		0.0 ± 1.2
Tronquart model	Muscle $\delta^{15}N = 1.01$ x fin $\delta^{15}N + 0.74$	0.92	<0.001		

Regression residuals

Fin $\delta^{13}C$ and $\delta^{15}N$ values were converted to muscle values using the species-specific and all-species models developed with data from this study, as well as models proposed by others. The species-specific and all-species model result in the smallest range of absolute residual values, followed by the general models proposed Tronquart and Jardine (Table 3). The all-species model developed with these fishes had similar residual values as the species-specific model. The other general models resulted in slightly greater residual ranges, but were only significantly different than the species-specific model for *M. ambigua* $\delta^{13}C$ values and *N. erebi* $\delta^{15}N$ values using the Jardine model, and *C. carpio* $\delta^{15}N$ values and *M. ambigua* $\delta^{15}N$ values using the Tronquart model. The Jardine model for *Nematalosa* spp. resulted in a greater range of residuals than the *N. erebi* specific model, but the difference was not significant (Table 3).

After fin $\delta^{13}C$ and $\delta^{15}N$ values were converted using species-specific and all-species models from this dataset, the converted $\delta^{13}C$ and $\delta^{15}N$ values were not significantly different from observed muscle values. However, all converted fin values using the Jardine model were significantly different ($P < 0.01$) than observed muscle values. Additionally, the *N. erebi* fin values converted using the Jardine model developed for *Nematalosa* spp. were also significantly different ($\delta^{13}C$: $P = 0.005$; $\delta^{15}N$: $P = 0.02$). Values converted using the Tronquart model were not significantly different with the exception of $\delta^{15}N$ for *M. ambigua* ($P < 0.001$).

Method considerations

The amount of powdered tissue required by the laboratory for analysis was 2 mg for carbon, and 0.4 mg for nitrogen. The use of a 5 mm biopsy punch consistently provided enough muscle tissue for one sample. The amount of sample collected from fin clips varied by species. The minimum size of fin clip needed was 1 cm^2. This size provided the dry weight necessary for analysis but did not always provide tissue above the minimum amount required and any loss during sample preparation may result in too little material. The weight per area varied by species (unpublished data), with smaller-bodied and thinner-finned species having lower values. In the larger-bodied species 1 cm^2 provided a sufficient amount of sample but is likely too small to incorporate both ray and membrane tissue which may influence results.

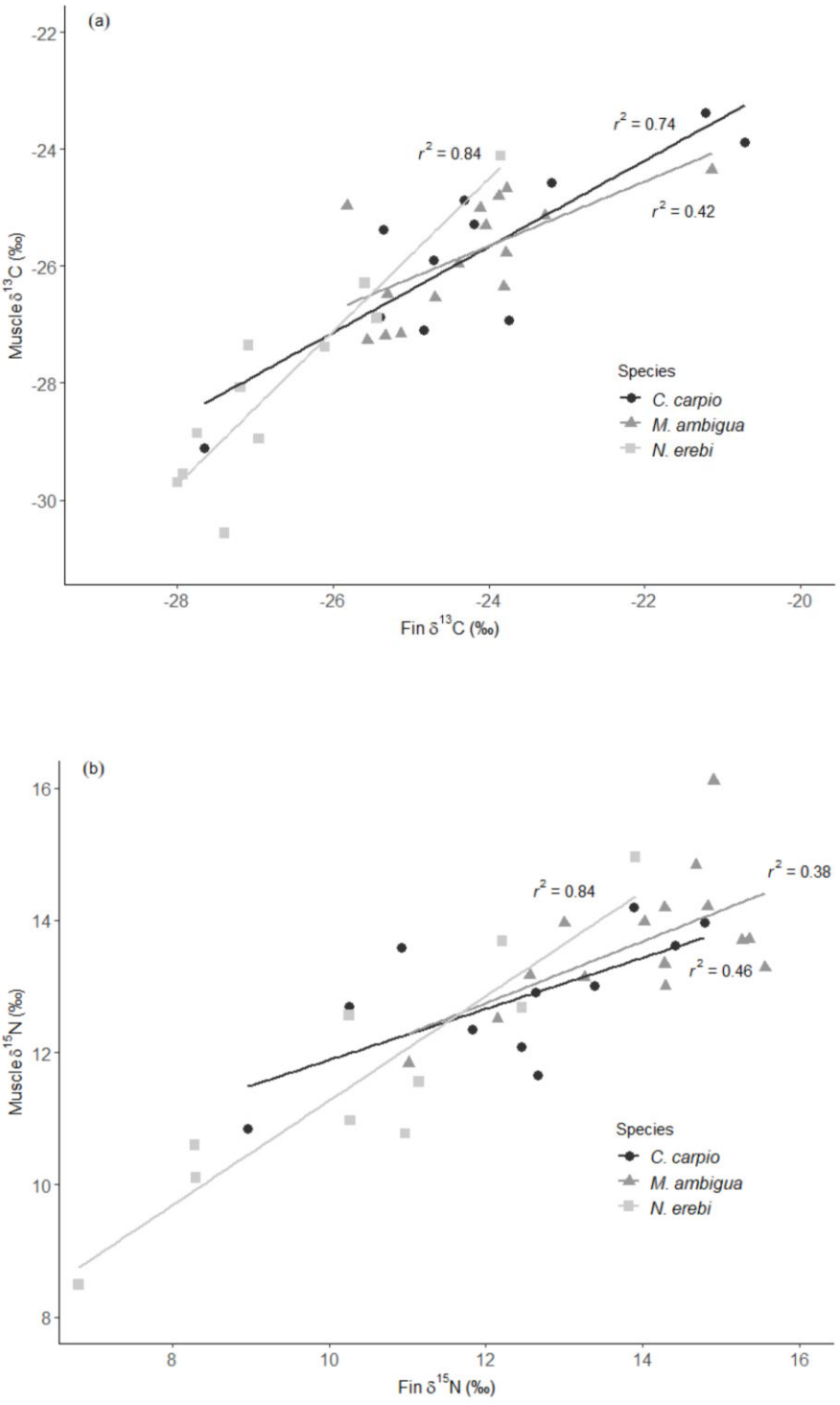

Figure 4-2 (a) $\delta^{13}C$ and (b) $\delta^{15}N$ in fin and muscle tissue for three species of fish from an Australian dryland river.

Table 4-3. Absolute residuals (mean ± s.d.) of tissue conversion equations for $\delta^{13}C$ and $\delta^{15}N$. The all-species equation represents combined data for the three species used in this study, additional regression equations are from Jardine *et al.* (2011) and Tronquart *et al.* (2012). *difference is significant from species-specific model ($p<0.05$).

Species		Species-specific	All-species	Jardine *Nematalosa*	Jardine general	Tronquart general
Cyprinus carpio	$\delta^{13}C$	0.70 ± 0.42	0.68 ± 0.50		1.10 ± 0.77	0.68 ± 0.48
Macquaria ambigua	$\delta^{13}C$	0.60 ± 0.42	0.61 ± 0.54		1.10 ± 0.59*	0.62 ± 0.50
Nematalosa erebi	$\delta^{13}C$	0.52 ± 0.49	0.68 ± 0.62	0.98 ± 0.78	1.05 ± 0.82	0.73 ± 0.64
Cyprinus carpio	$\delta^{15}N$	0.56 ± 0.47	0.58 ± 0.56		1.11 ± 1.00	1.25 ± 0.55*
Macquaria ambigua	$\delta^{15}N$	0.61 ± 0.48	0.60 ± 0.51		0.92 ± 0.58	1.23 ± 1.00*
Nematalosa erebi	$\delta^{15}N$	0.64 ± 0.33	0.73 ± 0.43	1.00 ± 0.76	1.43 ± 0.75*	0.79 ± 0.48

4.5 Discussion

We compared the $\delta^{13}C$ and $\delta^{15}N$ values of non-lethally collected dorsal muscle and caudal fin tissue to establish if there is a consistent relationship between the two tissue types. We found that $\delta^{13}C$ values of caudal fin and muscle tissue were significantly different for all three species and $\delta^{15}N$ values of tissues were significantly different for one species. The isotopic relationship between tissue types can be used to develop a tissue conversion model, allowing non-lethally collected fin tissue to be converted to equivalent muscle tissue values for inclusion in food web analysis. However, a small number of fish may need to be sacrificed to establish a species- or region-specific conversion model.

Overall, fin tissue was enriched in ^{13}C relative to muscle tissue but the relationship was predictable, with two of the three species studied here having a strong correlation. Fin tissue having higher $\delta^{13}C$ values was consistent with results in temperate, tropical, and reef fishes (Busst *et al.* 2015; Jardine *et al.* 2011; Willis *et al.* 2013; Winter *et al.* 2019). This consistent relationship indicated that fin tissue can be used as a proxy for muscle tissue in trophic studies. While the relationship was consistent,

differences in $\delta^{13}C$ values between tissues are large enough to influence assessment of resource use (Hanisch *et al.* 2010). For example, the mean difference was greater than the commonly used trophic fractionation rate of 0.4 ± 1.3‰ (Post 2002), and may alter ecological interpretation of results of trophic studies. A conversion factor should be applied to equate fin to muscle values to reduce the amount of variation.

The relationships between $\delta^{15}N$ in the two tissues was less clear, with only one of the species studied here having significantly different isotopic values. There was no consistent trend of enrichment, with fin tissue enriched in ^{15}N relative to muscle tissue for only one species. This lack of a consistent relationship was also found in temperate, tropical, and reef fishes (Galván *et al.* 2015; Hanisch *et al.* 2010; Jardine *et al.* 2011; Willis *et al.* 2013), and varies by species. Jardine *et al.* (2011) found that the average difference between fin and muscle $\delta^{15}N$ in Australian tropical species was negligible, recommending that a conversion factor is not necessary to use fin in place of muscle tissue in trophic studies. While the difference in $\delta^{15}N$ values between tissues in some species may not be great enough to influence assessments of resource use, the lack of a consistent relationship between tissues suggests that the species-specific tissue relationships should be examined before determining if a conversion factor is necessary when using fin as a surrogate for muscle.

Sources of variation

Examining the mechanisms that explain differences in the isotopic composition of tissues is outside the scope of this study. However, it is important to recognize the wide array of factors that have the potential to influence $\delta^{13}C$ and $\delta^{15}N$ in different tissues. White muscle tissue is favoured for many isotopic studies of trophic relationships in fish because it has low within tissue variability (Pinnegar and Polunin 1999; Suzuki *et al.* 2005), and has been well studied in fish and other vertebrates (Boecklen *et al.* 2011; Vander Zanden *et al.* 2015). Using a tissue which has not been well studied, such as caudal fin, introduces additional sources of variation which are important to consider. Here we briefly discuss potential factors influencing the isotopic composition of fish fin and muscle tissue.

The use of fin tissue as a proxy for muscle tissue assumes similar rates of isotopic turnover and incorporation into tissues. Isotopic turnover is influenced by both growth and catabolism, with relative contributions of each varying based on individual size, growth rate, tissue type, and potentially element type (Martínez del Rio and Carleton 2012; Reich *et al.* 2008; Thomas and Crowther 2015; Wolf *et al.* 2009). In individuals experiencing active growth, such as juveniles, growth contributes to turnover more than catabolism; whereas metabolic replacement is more important when growth is low (Weidel *et al.* 2011). Metabolic turnover rates vary based on tissue type and are influenced by physiological condition (Martínez del Rio and Carleton 2012; Perga and

Gerdeaux 2005). Studies in fish have found that tissue turnover time varies among species, and there is no consistency in which tissue has the higher turnover rate (Busst and Britton 2017; Heady and Moore 2013; McIntyre and Flecker 2006; Suzuki *et al.* 2005; Watanabe *et al.* 2005). Additionally, isotopic routing and differences in amino acid profiles among tissues influence isotopic composition of tissues (McMahon *et al.* 2010; McMahon *et al.* 2015). These sources of variation are difficult to account for and affect both muscle and fin tissue (MacNeil *et al.* 2006). We did not estimate turnover rates or relative contributions of growth or metabolism for the species used in this study, but it is reasonable to expect these processes to contribute to differences in isotopic composition between tissues.

Molecular composition of tissues may also influence isotope values. The %C and %N were consistently lower in fin tissue than muscle tissue indicating differences in molecular composition. Lipids are depleted in ^{13}C relative to protein (DeNiro and Epstein 1977; DeNiro and Epstein 1978), and the higher lipid content of muscle has been suggested as an explanation for differences in $\delta^{13}C$ values between tissues (Pinnegar and Polunin 1999). However, the C:N ratios of muscle and fin tissue were similar for the three fish species used in this study. Additionally, the lipid correction factor that was applied to both tissues reduced the influence of any potential lipid differences (Logan *et al.* 2008; Post *et al.* 2007). Therefore, it is unlikely that lipid content alone can explain the consistent differences in $\delta^{13}C$ values for these fishes.

Fish fins are composed of rays interspersed with skin, this composite of tissue types may introduce more variation in isotopic values than homogenous muscle tissue. Hayden *et al.* (2015) found that there was isotopic variation within fins associated with the proportion of ray and membrane in each section of fin, suggesting different isotopic turnover rates between ray and membrane. Unlike for muscle tissue, the fin section used for isotopic analysis is not standardised. The composition of fin sample used in previous studies varies from only skin (Willis *et al.* 2013), to membrane and soft rays (Galván *et al.* 2015; German and Miles 2010), or is not specified (Tronquart *et al.* 2012). Fin clips used in this study always consisted of both ray and membrane. Variation was minimised by collecting only the tip of the fin, but the proportions of ray and membrane varied among samples.

While there are likely differences in turnover rates of fin tissue components (Vander Zanden *et al.* 2015; Wolf *et al.* 2009), differences between tissue components alone do not explain the isotopic relationship between tissues. Willis *et al.* (2013) included only fin membrane for two different species and found that fin tissue for both species had higher $\delta^{13}C$ values than muscle tissue, but only one species had higher $\delta^{15}N$ values. A similar pattern to what was found for the three species used in this study. In addition to different turnover rates between ray and membrane, Hayden *et al.* (2015)

also found that growth and regeneration influenced $\delta^{15}N$, potentially helping to explain the inconsistent relationship for N. The isotopic relationship among tissues is complicated and warrants further investigation to better understand the mechanisms that determine isotopic composition and differences between species and tissues. Despite the differences, this and other studies have found strong relationships between tissue types.

Tissue conversion models

Caudal fins are a viable non-lethal alternative to muscle tissue when the additional error introduced is acceptable for the aims of a study. Some studies have found that in certain cases fins can be used directly in place of muscle tissue, because they exhibit similar trophic changes (Hanisch *et al.* 2010; Sanderson *et al.* 2009) or because differences are negligible (Jardine *et al.* 2011). However, the use of correction factors reduces error (Tronquart *et al.* 2012) and makes fin values more directly comparable to studies using muscle tissue (Sanderson *et al.* 2009).

The regression models developed here to convert isotopic values between types of tissue were comparable to those developed in other studies. The regression models for predicting $\delta^{13}C$ muscle values from $\delta^{13}C$ fin values had r^2 values ranging from 0.42 to 0.84. Models for other species had r^2 values between 0.44 and 0.99 (Finlay *et al.* 2002; Hanisch *et al.* 2010; Jardine *et al.* 2011; Sanderson *et al.* 2009; Tronquart *et al.* 2012). The regression models for predicting $\delta^{15}N$ muscle values from $\delta^{15}N$ fin values had r^2 values ranging from 0.38 to 0.84, other studies have found a range between 0.10 and 0.99 (Finlay *et al.* 2002; Hanisch *et al.* 2010; Jardine *et al.* 2011; Sanderson *et al.* 2009; Tronquart *et al.* 2012). In this study and others (reviewed in Willis *et al.* 2013) the isotopic relationship between fin and muscle tissue rarely exhibits a 1:1 slope, indicating that turnover rates vary between tissues.

There are important considerations for the development and use of regression models to convert fin isotopic values to equivalent muscle isotopic values. Size-related trends in the differences between tissue isotope values have been observed (Galván *et al.* 2015; Jardine *et al.* 2005; Jardine *et al.* 2011; Sanderson *et al.* 2009). While there was no significant influence of size on the linear models for the three species in this study, the sizes of fish included did not encompass the total potential size range. The isotopic relationships between tissue types can vary among different populations of the same species (Graham *et al.* 2013; Kelly *et al.* 2006; Vašek *et al.* 2017). The regression equations for *N. erebi* in this study, and those developed by Jardine *et al.* (2011) for *Nematalosa sp.* had different slopes and intercepts, indicating differences in the isotopic relationship between tissues. Indeed, the species-specific model for *Nematalosa sp.* from Jardine was a poor fit for *N. erebi* collected in this study. The tissue conversion model developed for these species may not be applicable to

populations in other locations (Galván *et al.* 2015), particularly if populations have a different range of $\delta^{13}C$ and $\delta^{15}N$ values. Fishes in Australia and New Guinea have documented muscle isotopic values ranging from -33.7‰ to -18.5‰ for $\delta^{13}C$ and 5.5‰ to 15.5‰ for $\delta^{15}N$ (Bunn *et al.* 2013). The range of isotopic values found here was -30.57‰ to -23.38‰ for $\delta^{13}C$ muscle tissue and -27.99‰ to -20.72‰ for $\delta^{13}C$ fin tissue; 8.49‰ to 16.11‰ for $\delta^{15}N$ muscle tissue and 6.81‰ to 15.56‰ for $\delta^{15}N$ of fin tissue. Low sample sizes in this study likely do not incorporate the range of natural variation for these populations.

Establishing species-specific tissue relationships requires sacrificing a small number of fish, making the prospect of developing a general tissue conversion model enticing. We converted the fin isotope values from the fish in this study using general models proposed by Tronquart *et al.* (2012), developed using temperate European fish species, and Jardine *et al.* (2011), developed using tropical Australian species, as well as a model developed using the three species from this study. The all-species model developed with these species had similar residual values as the species-specific model. The other general models resulted in slightly greater residual ranges. Fin values converted using the Jardine general model were all significantly different than observed muscle values, indicating that despite residuals from the regression equations not all being significantly greater, this model is not a good fit for these fish. Fin values converted using the Tronquart model were only different than observed muscle values for *M. ambigua* $\delta^{15}N$ values, suggesting that this model is a better fit despite being developed using European species. However, pooling data from multiple species and locations biases the slope of the regression towards one, when the true relationship between tissues is often not 1:1 (Galván *et al.* 2015; Willis *et al.* 2013), potentially influencing interpretation of the results of further analysis.

General models were tested by Busst *et al.* (2015) and Tronquart *et al.* (2012), who also found that species-specific models are most accurate but regional models still provide meaningful results. Developing a global model that would be applicable to all fish species is unlikely, and a species-specific model is ideal. However, when sacrificing a number of fish to develop a relationship is impractical, such as when working with rare species or in areas with low population density, a region-specific general model is still a decent predictor. The models developed in this study were not influenced by size differences or worsening drought conditions between sample collection events, and the region-specific general model was a good predictor for these fish. These results warrant further exploration of the development of region-specific general models.

Method considerations

The amount of fin tissue required for isotopic analysis is small, and collection has minimal impact Minimum size of fin clip needed for these species was one cm^2. A similar estimate was made by Galván *et al.* (2015) with slightly larger estimate of 2.5 cm^2 by Jardine *et al.* (2011). The collection of fin tissue is easy, requires no special equipment, and adds minimal additional handling time to taking basic measurements. Another logistical consideration is the volume of material that needs to be collected and transported. Field preservation of whole fish when working in relatively remote locations is impractical, particularly when dealing with large-bodied species.

When objectives of a study require the use of muscle tissue, biopsy plugs are a potential non-lethal option. The use of small muscle plugs may introduce slightly more variation in $\delta^{13}C$, but have been found to reflect whole organism isotope ratios (Schielke and Post 2010). Hanisch *et al.* (2010) used biopsy plugs to compare muscle and fin isotope ratios and had results comparable to studies using large muscle fillets. The procedure has been shown to not add additional long-term stress under controlled conditions (Henderson *et al.* 2016), and to have no effect on survival (Baker *et al.* 2004; Hanisch *et al.* 2010) even for fish as small as 110 mm (Henderson *et al.* 2016). Muscle biopsies of live fish are relatively easy to collect. Collection of biopsy plugs does add some additional handling time and increases potential for infection which may not be ideal for vulnerable populations.

4.6 Conclusion

Caudal fin tissue can be used as a non-lethal proxy for dorsal muscle tissue in food web studies. Conversion factors may need to be applied to compare fin isotopic values to those of muscle, but should be assessed for individual species. The use of species-specific conversion models is ideal, but region-specific general models provide similar results. This study has established conversion models for three species common in Australian lowland rivers. Fishes represent an important component of aquatic food webs, but standard methods for studying food webs rely on muscle tissue collected through invasive or lethal means. These findings will facilitate the inclusion of fishes in food web studies while minimising impact to the greater community by avoiding the removal of top consumers and species of low abundance. When non-lethal options are available that satisfy the same research requirements as lethal methods, it is incumbent upon researchers to minimise impact on the population by selecting the non-lethal option.

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Food web structure of fishes in the Barwon-Darling River

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5.1 Abstract

Water resource development to meet human water needs has altered the natural variability of river flow regimes. The Barwon-Darling River in the upper Murray-Darling Basin in Australia is a dryland river characterized by extreme hydrological variability that has experienced a change in hydrology and geomorphology since European settlement. We investigated the fish-centred food web structure in six sites in the main river channel to determine if varying levels of hydrological connectivity influenced trophic interactions. Samples were collected during a period of no flows when the river had receded into a series of disconnected waterholes. Stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) were used to determine consumer trophic position and community metrics. There were high levels of omnivory at all sites and indications of spatial variation with some sites showing more trophic overlap. However, no consistent pattern was found between food web structure and classifications of hydrological character. Due to the extended no-flow period, local habitat characteristics likely had a larger influence on food web structure than antecedent hydrology. Explaining spatial variation among sites likely requires a more thorough assessment of food web structure and site variables than this study allowed.

5.2 Introduction

Hydrological connectivity facilitates the movement of organisms, energy, and matter throughout river networks (Pringle, 2003; Zeug & Winemiller, 2008). Connectivity of the river network is dictated by a river's flow regime which drives fundamental ecosystem processes and sustains ecological integrity (Bunn & Arthington, 2002; Jardine, Bond, et al., 2015; Poff et al., 1997). Natural variability of the flow regime, including periods of disconnection, plays an integral role in shaping river ecosystems (Biggs, Nikora, & Snelder, 2005; Bunn, Thoms, Hamilton, & Capon, 2006; Greet, Webb, & Downes, 2011; Sheldon & Thoms, 2006). Varying levels of hydrological connection create and maintain a dynamic and heterogeneous environment (Amoros & Bornette, 2002; Frissell, Liss, Warren, & Hurley, 1986).

All rivers exhibit natural flow variability (Poff et al., 1997; Walker, 1985), but it is particularly pronounced in dryland rivers, which experience some of the highest levels of variability (Puckridge, Sheldon, Walker, & Boulton, 1998). However, most studies of river systems have occurred in temperate climates, which have predictable variability in hydrology linked to seasons (Junk, Bayley, & Sparks, 1989; Vannote, Minshall, Cummins, Sedell, & Cushing, 1980). The natural flow regime of intermittent dryland rivers is characterised as being intermittent and unpredictable (Powell, 2009;

Walker, Sheldon, & Puckridge, 1995), and creates enhanced natural fragmentation (i.e. periods of hydrological disconnection) (Puckridge, Walker, & Costelloe, 2000; Sheldon & Fellows, 2010) in comparison to temperate rivers. This highly variable connectivity is an integral part of ecosystem function within dryland rivers (Sheldon, Boulton, & Puckridge, 2002; Walker et al., 1995).

Flow regimes and habitat templates shape life histories of riverine biota (Lytle & Poff, 2004; Mims & Olden, 2012). Communities within intermittent dryland rivers have adapted strategies to cope with a highly variable flow regime, where the river alternates between periods of hydrological connection and periods of disconnection when the river contracts to isolated waterholes (Lytle & Poff, 2004; Mims & Olden, 2012; Sheldon et al., 2010). During these low- or no-flow periods, refugial waterholes enable biota to persist until the next period of hydrological connectivity (Arthington, Balcombe, Wilson, Thoms, & Marshall, 2005). Connection events prompt biota to move out of waterholes to exploit new resources (Marshall et al., 2016), and periods of isolation require mechanism to cope with declining environmental conditions (Arthington, Olden, Balcombe, & Thoms, 2010). Movement of organisms within the river network during periods of connection influences species composition and thus interactions within small habitat patches during periods of disconnection (Woodward & Hildrew, 2002). Environmental conditions and species interactions within these habitat patches then influence communities when flows reconnect habitats.

Food webs reflect species interactions and energy pathways within the biotic community (Cross et al., 2013; Vander Zanden, Chandra, Allen, Reuter, & Goldman, 2003), making them an ideal feature of ecosystems to examine when exploring the role of connectivity as a driver of ecosystem structure and function. Food webs are community indicators that integrate responses at a range of spatial and temporal scales (reviewed in Robson et al., 2017), and are influenced by hydrological connectivity. For example, frequency of connectivity is associated with basal resource use and food web structure (Reid, Delong, & Thoms, 2012) while predictability of flow influences trophic composition (Poff & Allan, 1995). Dietary specialization tends to be more prevalent when food sources are predictable, with omnivory and opportunism more common when flows are variable and access to resources is unpredictable (Blanchette et al. 2014, Pusey et al. 2010). The structure of food webs reflects basal scale interactions which form the foundation of ecosystem processes and provide a meaningful indication of community interactions (Thompson & Townsend, 2005a).

Stable isotopes are commonly used as a tool to characterise food web structure (Peterson & Fry, 1987; Post, 2002b). Food resources tend to have different ratios of ^{13}C to ^{12}C ($\delta^{13}C$) and ^{15}N to ^{14}N ($\delta^{15}N$), allowing food resource assimilation of consumers to be inferred from the isotopic signatures

in their tissues (Fry, 2006). Consumers preferentially conserve the heavier isotope, resulting in them being enriched with heavier isotopes in relation to their food (DeNiro & Epstein, 1978, 1981). Isotopes of C are relatively conserved across trophic levels (Post, 2002b), and primary producers generally have distinct ^{13}C signatures, making $\delta^{13}C$ useful to trace carbon flow pathways (DeNiro & Epstein, 1978; Perkins et al., 2014). Enrichment of N through consumers, is considered predictable with each energy transfer, enabling N isotope ratios to be used to estimate trophic positions (Post, 2002b). Isotope ratios can also be used as a measure of isotopic niche space (Bearhop, Adams, Waldron, Fuller, & MacLeod, 2004; Newsome, Martinez del Rio, Bearhop, & Phillips, 2007). Layman, Arrington, Montaña, and Post (2007) proposed a set of metrics to quantify the dispersion of $\delta^{13}C$ and $\delta^{15}N$ in bivariate space, representing measures of community isotopic diversity, redundancy, and overall resource distribution. These measures provide continuous metrics to compare food web complexity across sites (Layman et al., 2012).

Food webs in streams and rivers in arid environments differ from those of temperate climates (Bunn, Balcombe, Davies, Fellows, & McKenzie-Smith, 2006). In intermittent dryland rivers, which have unpredictable and variable hydrology, autochthonous sources of carbon are thought to be more important than in temperate rivers, fishes exhibit little specialised feeding, and communities are less diverse (Bunn, Balcombe, et al., 2006). High rates of omnivory and condensed trophic links are also common in rivers with variable hydrology (Blanchette et al., 2014; Douglas, Bunn, & Davies, 2005; Poff & Allan, 1995). In Australian arid and seasonal tropical floodplain rivers benthic algae has been identified as the dominant energy source for larger consumers in waterholes (Balcombe, Bunn, McKenzie-Smith, & Davies, 2005; Bunn, Davies, & Winning, 2003), but fish may subsidise their diet with floodplain carbon during periods of connection (Jardine et al., 2013). However, contrasting studies have found that non-algal energy sources such as leaf litter and phytoplankton are important for sustaining fish in main channel habitats, with no evidence that fish transport carbon from the floodplain (Beesley et al., 2020; Pusey et al., 2020). The importance of different basal energy sources is spatially and temporally variable (Leigh, Burford, Sheldon, & Bunn, 2010); and within the same river, energy sources supporting food webs can vary among habitat patches (Blanchette, Davis, Jardine, & Pearson, 2014). Consumers likely exploit all available energy sources at different life stages and in different environmental conditions (Jardine, Woods, et al., 2015). Changing hydrological connectivity through water resource development changes connectedness among habitat patches, affecting the flow of nutrients and organisms throughout the ecosystem. Changing connectivity thus has the potential to alter food web structure.

A retrospective study of the Barwon-Darling River, a dryland river in Australia, used stable isotope derived metrics to examine the influence of food web structure by comparing 100 km segments in the upper and lower river between two time periods, pre- and post-regulation (Thoms & Delong, 2018). This study found that mean trophic position of three of the four fish feeding guilds assessed and food chain length both increased post-regulation but were not different between upper and lower river segments (Thoms & Delong, 2018). They also found a slight increase in the contribution of pelagic basal sources and lower community niche space, increased trophic redundancy, and a more even distribution of trophic niches post-regulation. However, this study assessed food webs at a large temporal scale and aggregated individuals from a large spatial scale, which may not reflect smaller-scale processes that structure food webs within habitat patches (Thompson & Townsend, 2005b). We still lack an understanding of smaller scale food web structure of fishes within the main river channel and how it may be influenced by altered hydrological connectivity. This study aims to help fill that knowledge gap.

Aim

The aim of this study was to determine how contrasting connectivity regimes structure fish-centred food webs within the main channel of a large dryland river in Australia. First, flow gauge data were used to characterise hydrological connectivity of study sites. These characterisations were then used to compare differences in food web metrics among sites with variable hydrological character. It was expected that there would be minimal spatial variation in food web structure due to high omnivory and low species diversity, but patterns in small spatial variation would be related to altered hydrology and geomorphology of the river. Specifically, that sites with greater hydrological connectivity will have lower trophic overlap, greater trophic area, and longer food chain length (FCL) reflecting a larger number of trophic transfers. Increasing hydrological isolation of sites will increase trophic overlap, reduce trophic area, and have shorter FCL due to higher rates of omnivory and competition for limited resources (McHugh, McIntosh, & Jellyman, 2010; Post & Takimoto, 2007; Takimoto & Post, 2013; Thompson & Townsend, 2005a).

Study area

The Barwon-Darling River in New South Wales, Australia is within the upper Murray-Darling Basin. It is a low gradient river flowing for much of its length through arid and semi-arid landscape. It has a catchment area of 699,000 km^2, 60% of which is less than 300 m above sea level, and a main channel

length of 3,100 km (Boys and Thoms 2006). This river has one of the most variable flow regimes in the world (Puckridge et al., 1998), but exists for most of the time in a low-flow stage when water is restricted to the main channel (Thoms & Sheldon, 2000; Thoms, Sheldon, & Crabb, 2004). During periods of extended drought surface water in the main channel contracts to disconnected waterholes (Walker et al., 1995).

The Barwon-Darling River has undergone extensive modification since the 1960s with increasing water extraction for irrigation, nine headwater dams, and 15 low-level weirs within the main channel (NSW DPI, 2019). This water resource development has produced changes in both the hydrology and geomorphology of the river. Prior to regulation, the river flowed 92% of the time and periods of no flow were short (Mallen-Cooper & Zampatti, 2020). Post-regulation of the river, the magnitude of near-annual flow pulses has been reduced by over 90%, resulting in longer durations of lentic conditions, while the spatial extent of lotic conditions has been limited during droughts due to weir pools (Mallen-Cooper & Zampatti, 2020). A change in hydrological character was most evident in low-flow periods post-2000. Upstream sections of the river have experienced more frequent, but shorter, periods with flows low enough to disconnect habitats, while sections downstream of Bourke experience fewer total dry spells but the duration of dry spells are longer (MDBA, 2018). Water storage and extraction for consumptive use exacerbates the impact of drought and has extended periods of lentic conditions beyond what has historically occurred (Mallen-Cooper & Zampatti, 2020).

The physical template of the Barwon-Darling River has also changed. In a comparison of historical and modern river profiles, Pearson, Reid, Miller, and Ryder (2020) found that in areas influenced by weir pools the depth of waterholes had a median increase of 1.4 m and the distance between waterholes decreased by 21 to 98%. Deep waterholes in areas of weir influence have become more connected due to the stabilisation of water levels by weirs. They also found that in areas outside of weir pool influence the depth of waterholes had a median decrease of 1.6 m and the distance between deep waterholes has increased. Deep waterholes in areas outside of weir pool influence have become less connected. The magnitude of this effect was particularly pronounced in sections upstream of Bourke (Figure 1) (Pearson et al., 2020).

Aquatic ecosystems in the Barwon-Darling River have also been affected by river regulation. There are only about 46 native species of freshwater fish that have been described in the Murray-Darling Basin (Lintermans, 2007) with even fewer found within the Darling River (Boys, Esslemont, & Thoms, 2005; Gehrke, Brown, Schiller, Moffatt, & Bruce, 1995). It is estimated that native fish populations are only about 10% of their pre-European settlements levels, and biomass in the Murray-Darling

Basin is dominated by non-native invasive species (MDBA, 2004). Comparisons of fish abundance between regulated and unregulated reaches indicated that there was a reduced abundance of native species in regulated reaches, and a greater abundance of juveniles in unregulated reaches (Gehrke, 1997). Increased river regulation corresponded to a reduction in species diversity and an increase in non-native species (Gehrke et al., 1995).

5.3 Methods

Samples were collected from six sites along a 1,390 km stretch of the Barwon-Darling River in New South Wales between Mungindi and Menindee, NSW (Figure 1, Figure 2). Samples were collected during a drought when the river had experienced several extended cease-to-flow periods. With the exception of a small flow event near Collarenebri in November 2018, there was no flow in the river between July 2018 and the time of sample collection in March 2019 (https://realtimedata.waternsw.com.au/ [accessed Nov 2019]) (Figure 3). .

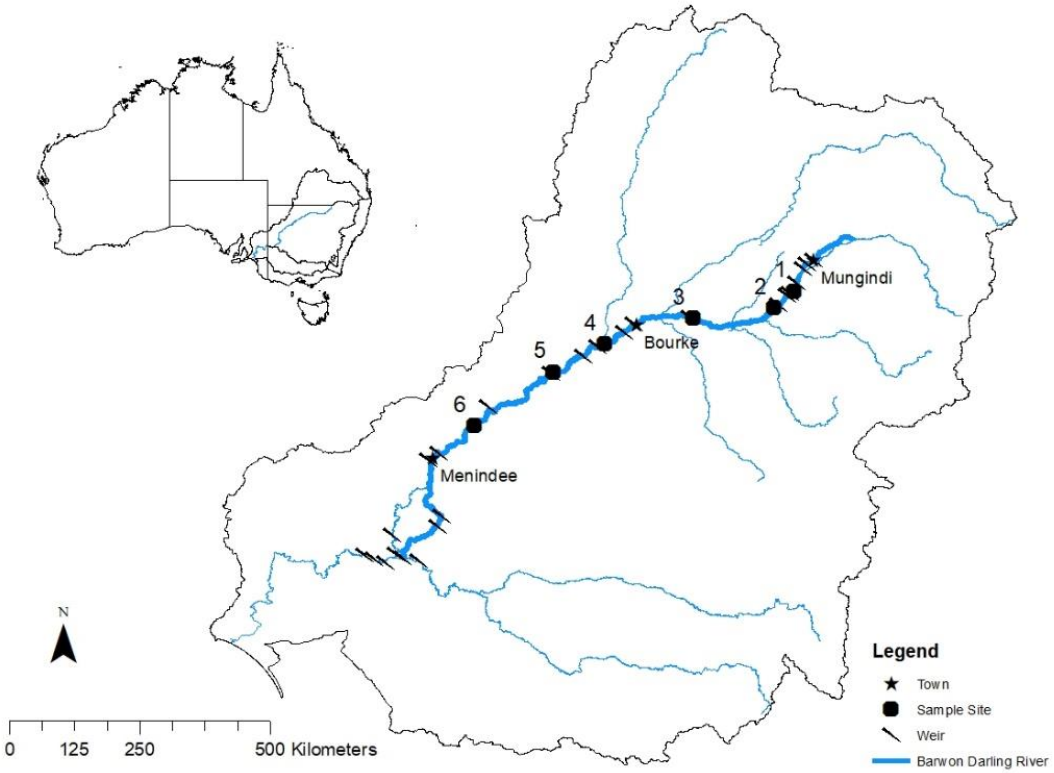

Figure 5-1 Location of sample collection sites in the Murray-Darling Basin, Australia

Characterisation of study sites

Sites were characterised based on flow frequency, size of site, and location in relation to weirs. Frequency and duration of cease-to-flow, baseflow, and large-fresh events were calculated using the Hydrostats package in R (Bond, 2015) based on data from gauging stations above and below sample sites (Table 1) (https://realtimedata.waternsw.com.au/) for a five-year period prior to sample collection. Flow values for sites 1 and 4 are the weighted average of upstream and downstream gauge values based on distance to gauge. Thresholds and definitions for flow classifications were taken from Sheldon (2019). Cease-to-flow is when there is partial or total drying of the river channel and surface water has contracted to disconnected pools. Baseflow is when there is connectivity between pools and riffles and along channels, depth is sufficient for fish to move along reaches. Large fresh events inundate benches and snags, support productivity and transfer of nutrients, and may connect some wetlands and anabranches. Sizes of sites are based on observations of site conditions at time of sampling and depth measurements taken at deepest point of pool, no other physical measurements were taken. Distance from sites to weirs was measured in the R package Riverdist v. 0.15.0 (Tyers, 2017) using spatial data obtained from the Australian Hydrological Geospatial Fabric v.2.1.1 (Bureau of Meteorology, 2014). These characterisations were used to rank sites according to their hydrological isolation.

Sample collection and preparation

Fish used in this study for stable isotope analysis were collected opportunistically during sample collection for a larger study. In March 2019, fish were collected using standard boat and backpack electrofishing methods and baited and unbaited traps (MDBC, 2008). Electrofishing was conducted in the river channel edge and middle habitats, as well as any structure (e.g. snags) if present. Fish tissue for stable isotope analysis was removed from live fish following the method of McIntosh and Reid (2020). Tissue was taken from the distal portion of the upper caudal fin for all species but carp gudgeons, which were collected whole. Tissue collection was standardized to collect from the tip following suggestions of Hayden et al. (2015). At least 1 cm^2 of tissue including both membrane and ray was collected from small individuals, with more tissue collected from larger fish. Attempts were made to collect samples from fish within the same size class to avoid any ontogenetic related feeding behaviour influence on interpretation of results (Davis, Blanchette, Pusey, Jardine, & Pearson, 2012; Pusey et al., 2020). Samples were not collected from juveniles, but it was not possible to restrict samples to certain size classes. We attempted to collect at least three individuals of each species, however at some sites this was not possible due to scarcity of catch. One golden perch in site 4 was collected from a nearby site, and only one golden perch was used in analysis for site 3 (Table 3). Samples were held on ice, rinsed with distilled water, and frozen within 24 hours of

collection. The head and digestive tract were removed from carp gudgeons prior to isotope analysis. Samples were taken from live fish which were released at the site of collection, except for carp gudgeons and some common carp which were humanely euthanised. The collection of animals used in this study complied with University of New England's animal research authority AEC 18-051 and NSW DPI ACEC Permit No. 98/14.

Figure 5-2 Sample collection sites in the Barwon-Darling River, Australia. March 2019.

Long-lived consumers were selected to represent basal resource isotope signatures (Post, 2002b). Live mussels were collected when available, however they were not found at all sites. The mussel sample from site 2 was collected from a nearby site. Tissue was separated from the shell, rinsed with distilled water, and frozen within 24 hours of collection. Adductor muscle tissue was used for isotope analysis. Two mussel samples were used for site 6. The remaining sites have one mussel sample but should be representative of site conditions and introduce minimal error (McKinney, Lake, Allen, & Ryba, 1999). Freshwater shrimp, *Macrobrachium australiense*, individuals were collected at each site, kept on ice and held in freshwater for several hours, and frozen within 12 hours of collection. The exoskeleton was removed prior to analysis. Samples represent composites of four to six of the largest individuals.

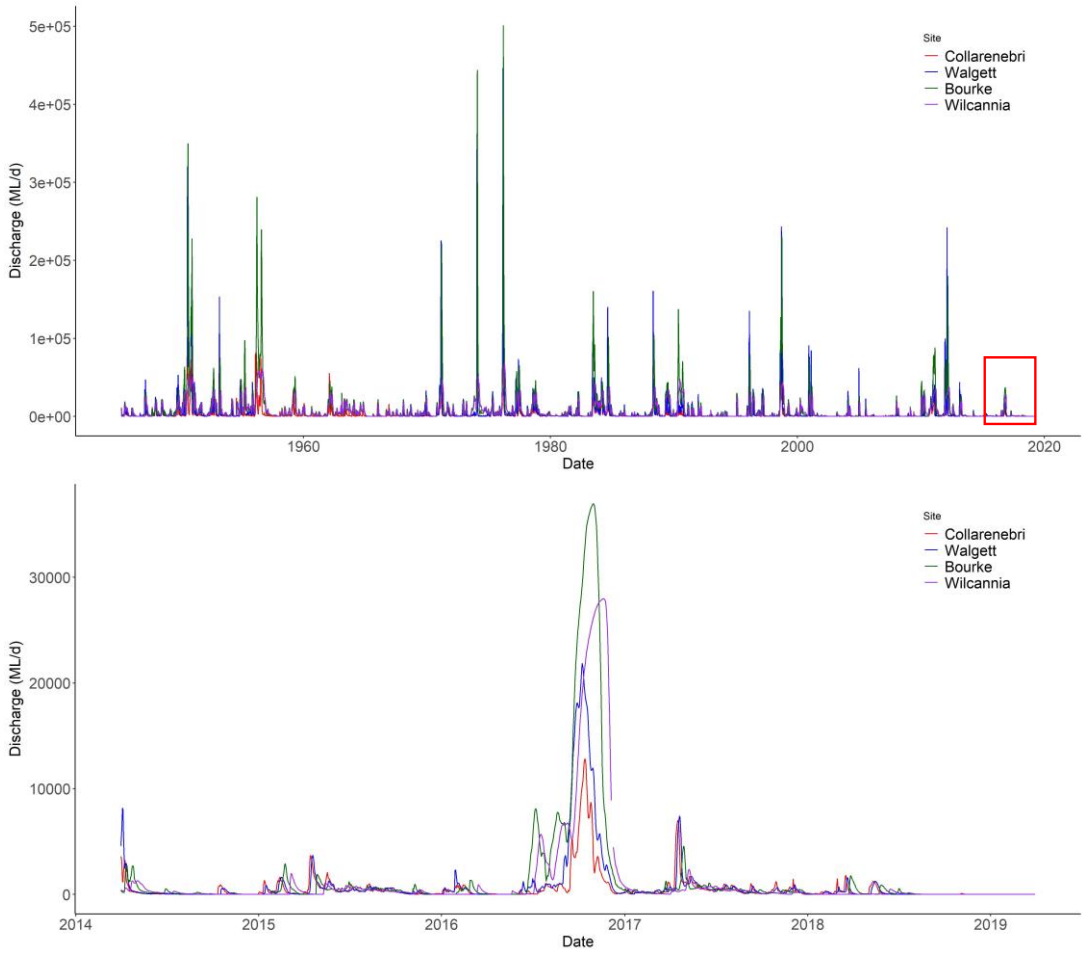

Figure 5-3 Daily flow data at four gauging stations along the Barwon-Darling River, Australia. The top hydrograph ranges from 1945 to 2019, the bottom hydrograph ranges from March 2014 to March 2019.

In the laboratory, tissue was dried at 60°C for 48 hours, ground to a fine powder and weighed into tin capsules. Carbon and nitrogen stable isotope ratios were analysed separately with 2 ± 0.05 mg of powder for carbon, and 0.4 ± 0.05 mg for nitrogen. Samples were combusted in a continuous flow isotope ratio mass spectrometer (Sercon 20-22, Sercon Limited, Cheshire, U.K.) at University of New England, Armidale, Australia. Each run included a laboratory standard every 20 samples to account for equipment drift and estimate error. $^{15}N/^{14}N$ was measured relative to atmospheric N standard and $^{13}C/^{12}C$ was measured relative to Vienna Pee Dee Belemnite standard. Isotope values are reported in the δ notation, defined as parts per thousand (‰) deviation from standard material. $\delta^{15}N$ or $\delta^{13}C = [(R_{sample}/R_{standard}) - 1] \times 1000$, where R = $^{15}N/^{14}N$ or $^{13}C/^{12}C$. Analytical precision was estimated to be 0.3‰ for $\delta^{13}C$ and 0.6‰ for $\delta^{15}N$. Lipid content was variable for all species with C:N ratio of some samples >4, all $\delta^{13}C$ values were corrected for lipids following Post et al. (2007).

Stable isotope analysis

Fin tissue isotopic values were converted to equivalent muscle tissue values following methods of McIntosh and Reid (2020) for all species but carp gudgeons. Species-specific tissue conversion equations were used for carp ($\delta^{13}C = 0.7360 \times \text{fin } \delta^{13}C - 8.0028$; $\delta^{15}N = 0.3869 \times \text{fin } \delta^{15}N + 8.0170$), golden perch ($\delta^{13}C = 0.5517 \times \text{fin } \delta^{13}C - 12.4129$; $\delta^{15}N = 0.4671 \times \text{fin } \delta^{15}N + 7.1422$), and bony bream ($\delta^{13}C = 1.3086 \times \text{fin } \delta^{13}C + 6.9075$; $\delta^{15}N = 0.7904 \times \text{fin } \delta^{15}N + 3.3677$). A regional tissue conversion equation was used for spangled perch and Murray cod ($\delta^{13}C = 0.8472 \times \text{fin } \delta^{13}C - 5.3096$; $\delta^{15}N = 0.5794 \times \text{fin } \delta^{15}N + 5.5924$).

Diet-tissue $\delta^{15}N$ fractionation estimates calculated by Bunn, Leigh, and Jardine (2013) for Australian fishes were used for all species except bony bream for which the species-specific fractionation rate used was taken from Pusey et al. (2020). Trophic enrichment factors were as follows: predatory fishes, 5.7 ± 1.6; omnivorous fishes, 4.3 ± 1.5; bony bream, 4.1 ± 1.3. Fish were assigned to feeding groups based on the literature (Khan, 2003; Pusey, Kennard, & Arthington, 2004). Bony bream were classified as detritivores; golden perch and Murray cod as predatory fishes; common carp, carp gudgeons, and spangled perch as omnivores.

The one baseline trophic position model of Post (2002b) was used to calculate individual trophic positions (TP) as: $TP_{consumer} = \lambda + (\delta^{15}N_{consumer} - \delta^{15}N_{base})/\Delta N$, where ΔN is trophic enrichment factor values of N and λ is the assumed trophic level of baseline, here $\lambda = 2$ for primary consumers. Primary consumers were not used to assess basal C sources, but their $\delta^{13}C$ values were used as an indication of the breadth of $\delta^{13}C$ values within sites. Mean TP for each site was calculated as the mean trophic position for the four consumers that were found in all sites. Food chain length (FCL) was defined as the number of energy transfers from the base to the top of the food web and was calculated as the mean trophic position of the top consumer species at each site (Post, 2002a). Within individual sites, Pearson correlation was used to test if there is a relationship between fish length and TP.

Isotope biplots of $\delta^{13}C$ and $\delta^{15}N$ were used to visually represent the food web space. Community trophic structure was quantified using the metrics proposed by Layman et al. (2007), based on the spread of $\delta^{13}C$ and $\delta^{15}N$ values in consumers. NR (nitrogen range) measures the vertical structure within a food web, and indication of the number of trophic levels and trophic diversity. TA (total area of the convex hull encompassing the community) is an indication of the total amount of niche space occupied, a reflection of the total extent of trophic diversity. CD (mean distance to the centroid) measures the average degree of trophic diversity and is less influenced by outlier species than TA. NND (mean nearest neighbour distance) measures density of species packing, determined by the proportion of species with similar trophic ecologies. SDNND (standard deviation of the nearest

neighbour distance) measures evenness of species packing and is less influenced by sample size than NND. Analysis only included the four species present at all sites, golden perch from site 3 was only represented by a single individual.

Trophic positions and stable isotope signatures of each fish species were compared across all sites with a one-way analysis of variance (ANOVA) in R (R Core Team, 2018). This test included only the four species that were present in all sites. Site 3 was removed for comparisons of golden perch as that site was represented by one sample. Assumptions were assessed by box plots to check for outliers, Shapiro-Wilk's normality test to check for normality, and Levene's test to check for homogeneity of variance. There were no extreme outliers, residuals were normally distributed ($p > 0.05$) and there was homogeneity of variances ($p > 0.05$). All pairwise comparisons were assessed between sites for each species with Tukey's HSD test (significance level of 0.05).

5.4 Results

The proportion of time that cease-to-flow events occur, and their duration, generally increased from upstream to downstream except at site 3 which had a longer duration than downstream sites and sites 5 which was disconnected for a smaller proportion of time than site 4 but a longer duration than site 6. The number of baseflow and greater events per year and their frequency varied. Sites 1, 2 and 4 experienced more frequent shorter flows than sites 3, 5, and 6. The average duration of large fresh events increased upstream to downstream, but the frequency of events varied (Table 1, Table 2).

Table 5-1 Flow data from gauging stations near sample collection locations in the Barwon-Darling River, Australia. Values for sites 1 and 4 represent the weighted mean value of data from upstream and downstream gauging station. Flow data range from March 2014 through March 2019.

Site	Distance to gauge (km)		Nearest gauge ID	Proportion time cease to flows occur	Average cease-to-flow spell duration (days)	Frequency of above baseflow events (no. per year)	Average duration of above baseflow events (days)	Frequency of high spell events (no. per year)	Average duration of spell events (days)	Cease-to-flow date
	Upstream	Downstream								
1	24.9	12.9	422004; 422003	0.35	25.60	6.25	21.43	0.98	17.41	9/11/2018; 18/06/2018
2	54.4	8.2	422025	0.31	46.67	5.00	21.48	1.75	17.29	19/06/2018
3	88.5	9.1	422002	0.32	65.44	3.75	44.93	1.25	22.60	29/07/2018
4	58.6	54.4	425035; 425004	0.38	60.74	6.69	30.10	1.16	32.47	30/07/2018
5	136.9	13.0	425900	0.35	70.56	3.75	42.87	0.50	46.00	2/08/2018
6	73.6		425008	0.41	61.75	3.25	55.45	0.75	40.67	10/08/2018

Table 5-2 Classifications of hydrological character based on flow data for the 5-year period prior to sample collection, and site characteristics. Trophic position (TP) and food chain length (FCL) based on $\delta^{15}N$ values.

Site	Location	Proportion of time site disconnected	Length of dry spells	Frequency of above baseflow events	Size of site (depth)	Weir influence?	Distance to weir (km)	Mean TP (s.d.)	TP range	FCL
1	148.662098; -29.515939	Medium	Low	High	Large (2.5 m)	Yes	Upstream 21 Downstream 14	2.43 (0.32)	0.85	2.72
2	148.316499; -29.805565	Low	Low	High	Small (multiple pools 0.5 m)	No	Upstream 15 Downstream (Site 3)	1.93 (0.31)	1.01	2.23
3	146.914483; -29.982426	Low	High	Low	Large (8 m)	Yes	Upstream (Site 2) Downstream 11	2.00 (0.56)	1.53	2.69
4	145.389831; -30.430003	High	Medium	High	Small (2.5 m)	No	Upstream 82 Downstream 21	2.39 (0.46)	1.2	2.89
5	144.508775; -30.910538	Medium	High	Low	Large (3 m)	Yes	Upstream 115 Downstream 9	1.95 (0.20)	0.61	2.17
6	143.141345; -31.836356	High	Medium	Low	Small (3 m)	No	Upstream 78 Downstream 147	2.39 (0.44)	1.16	2.78

Table 5-3 Fish size ranges and sample sizes collected in six sites in the Barwon-Darling River. Samples were collected in March 2019. Ranges of C and N elemental composition and stable isotope values. Trophic enrichment factors (T.E.F.) used to calculate trophic position for each species.

Species name	Common name	Fork length (mm)	*n*	C (%)	N (%)	C:N	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	T.E.F. (‰)
Cyprinus carpio	Common carp	283 – 670	18	18.96 – 32.82	6.03 – 10.27	2.66 – 4.72	-27.07 - -22.63	11.48 – 13.72	4.3 ± 1.5
Macquaria ambigua	Golden perch	145 – 498	16	14.62 – 33.99	3.59 – 9.38	2.90 – 4.21	-27.78 - -24.09	12.29 – 14.86	5.7 ± 1.6
Nematalosa erebi	Bony bream	121 – 297	18	13.83 – 20.66	4.05 – 7.28	2.80 – 4.32	-29.54 - -21.69	8.63 – 13.50	4.1 ± 1.3
Hypseleotris Spp	Western carp gudgeon	23 – 35	18	38.61 – 45.1	8.21 – 12.44	3.34 – 4.81	-28.09 - -18.37	6.62 – 12.69	4.3 ± 1.5
L. unicolor	Spangled perch	102 – 128	6	22.78 – 29.02	5.79 – 7.79	3.52 – 4.51	-26.50 - -24.93	10.92 – 13.70	4.3 ± 1.5
M. Peelii	Murray cod	539 – 918	4	25.94 – 36.66	8.77 – 11.65		-27.25 - -22.71	12.06 – 14.64	5.7 ± 1.6
All fish			70						
M. australiense			6	42.03 – 45.52		3.15 – 4.40	-25.97 - -19.02	8.61 – 12.9	
Bivalvia			7	44.27 – 46.30	11.53 – 14.5	3.16 – 3.93	-30.71 - -27.25	9.71 – 14.74	

Mussels and freshwater shrimp were used as indicators of basal resource isotope signatures. Mussel $\delta^{13}C$ ranged from -30.7 to -27.3‰ and shrimp $\delta^{13}C$ ranged from -26.0 to -19.0‰. This encompassed most of the fish $\delta^{13}C$ values which ranged from-29.5 to -18.4‰. $\delta^{15}N$ values of mussels ranged from 9.7 to 14.74‰ and shrimp ranged from 8.6 to 12.9‰. Fish $\delta^{15}N$ values ranged from 6.6 to 14.9‰ (Table 3). At all sites, mussel $\delta^{15}N$ signatures indicated that they do not represent the lowest consumer, but their $\delta^{13}C$ values at most sites were the least enriched. Mussels were thus not used in calculation of trophic positions but were used as an indication of pelagic basal resource $\delta^{13}C$ values. Shrimp were consistently more enriched in ^{13}C than mussels and were used as an indication of benthic $\delta^{13}C$ resource values. There was no species that was consistently the lowest consumer at all sites and shrimp were chosen as baseline consumers for fish TP calculations. Estimates of TP do not capture the range of basal resources available to consumers and freshwater shrimp likely do not occupy a discrete trophic level. Estimates of TPs should therefore be considered relative to these sites and not absolute measures of TP.

Isotope biplots provide an overall picture of food web structure at each site (Figure 4). Only four species, bony bream, golden perch, common carp, and carp gudgeons, were reliably caught at all sites in sufficient numbers for analysis. Spangled perch at sites 1 and 3, and Murray cod at sites 2 and 6, were included in isotope biplots but were not included in statistical analysis. All sites displayed a relatively condensed biplot, most notably site 5. The $\delta^{13}C$ range of mussels and shrimp encompass most of the fish range and provide some indication of basal resource use. At sites in the upper section of the river (sites 1, 2, and 3) fish track closest to shrimp, suggesting a greater reliance on benthic resources. At sites lower in the river fish $\delta^{13}C$ values are between the mussel and shrimp values, suggesting a reliance on both benthic and pelagic resources. Carp gudgeons had the least enriched $\delta^{15}N$ values at all sites but 5, where bony bream values were slightly lower. Golden perch had the highest $\delta^{15}N$ at all sites apart from site 2, where a Murray cod sample was included.

The community trophic structure of the four fish species present in all sites varied among sites. Site 5 had the smallest niche space (TA), trophic diversity (CD), and the most species packing (NND). Site 3 had the largest niche space and trophic diversity, it also had relatively low species packing with the most even distribution of trophic niches (SDNND) (Figure 4). Site 6 also had a large niche space and trophic diversity but less evenness of species packing. Sites 1, 2, and 4 were similar, but site 2 had the most uneven species packing with high trophic overlap.

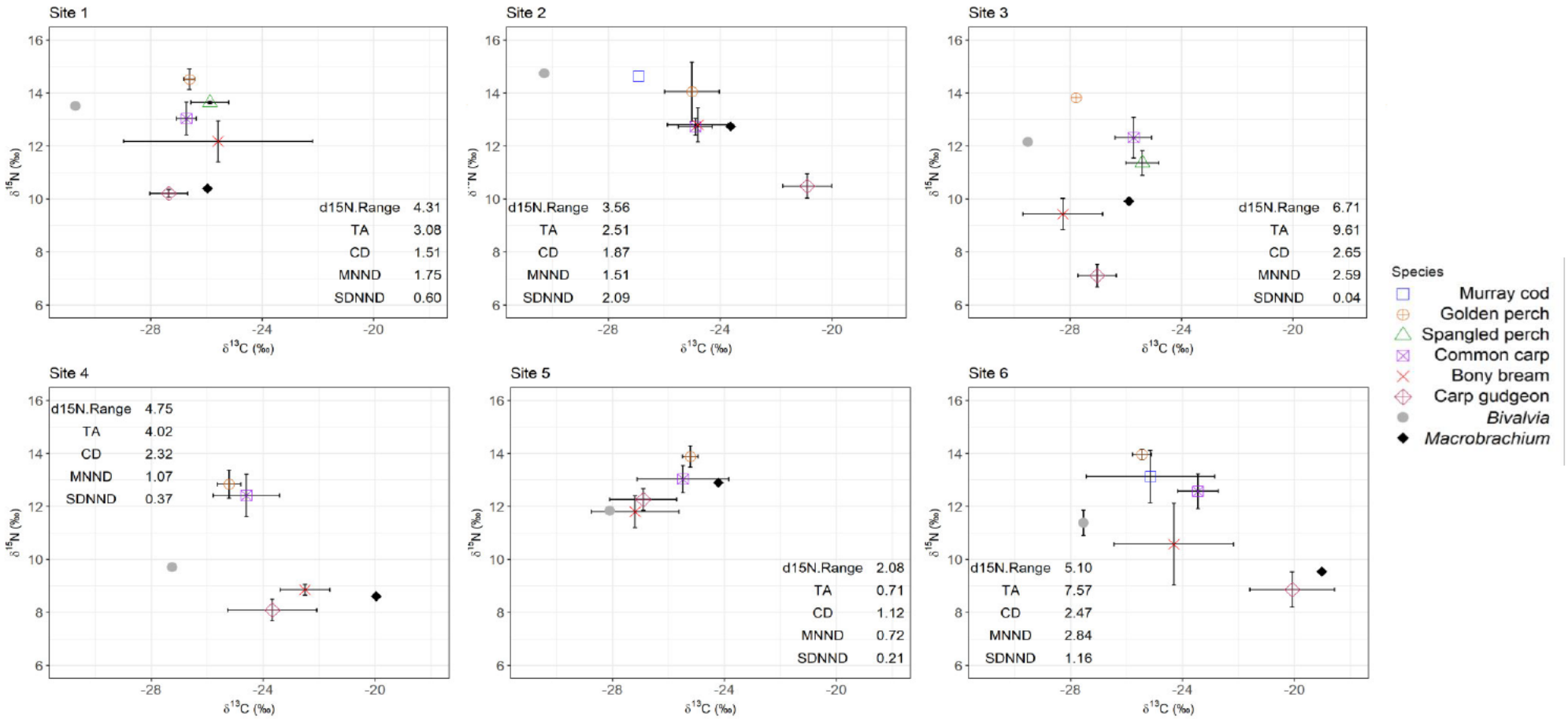

Figure 5-4 Stable isotope biplot and associated Layman metrics. Points represent mean (± 1 SD) $\delta^{13}C$ and $\delta^{15}N$ values for each species in each site. Samples were collected in March 2019 in the Barwon-Darling River. TA = trophic area; CD = centroid distance; MNND = mean nearest neighbour distance; SDNND = standard deviation of nearest neighbour distance.

Considering only the four species present at all sites there were significant differences in $\delta^{13}C$ values among sites, but no single site was significantly different from all the other sites. Golden perch $\delta^{13}C$ values were different among sites ($F_{(4,10)} = 4.476$, $p = 0.025$). Pairwise comparisons indicated that site 1 was the most different but was only significantly different from site 2. Bony bream $\delta^{13}C$ values were different among sites ($F_{(5,12)} = 3.404$, $p = 0.038$), but pairwise comparisons indicated the only difference was between sites 3 and 4. Common carp $\delta^{13}C$ values were different among sites ($F_{(5,12)} = 4.017$, $p = 0.023$), but the only difference was between site 1 and 6. Carp gudgeon $\delta^{13}C$ values were different among sites ($F_{(5,12)} = 24.131$, $p < 0.0001$) and were the most variable with 10 of 15 pairwise comparisons being significant (Figure S1).

There were also significant differences in $\delta^{15}N$ values among sites, but no single site was significantly different from all the other sites. Golden perch $\delta^{15}N$ values were not significantly different among sites ($F_{(4,10)} = 3.048$, $p = 0.07$), but pairwise comparisons found a significant difference between sites 1 and 4. Bony bream $\delta^{15}N$ values were different among sites ($F_{(5,12)} = 10.876$, $p = 0.0004$) and 6 of 15 pairwise comparisons were significant. Common carp $\delta^{15}N$ were not different among sites ($F_{(5,12)} = 0.697$, $p = 0.636$). Carp gudgeons $\delta^{15}N$ were different among sites ($F_{(5,12)} = 52.6$, $p < 0.0001$) and again were the most variable with 13 of the 15 pairwise comparisons being significant (Figure S2).

FCL and TPs of four common consumers varied among sites (Figure 5). FCL ranged from 2.17 to 2.89 (Table 2). Sites 2 and 5 had the shortest FCL, with golden perch as the top consumer. The longest FCL was at site 4 where common carp, an omnivore, was the top consumer. Mean TP varied from 1.93 at site 2 to 2.43 at site 1 (Table 2). The range of TPs spanned a full trophic level (i.e. from 2.0 – 3.0) at sites 2, 3, 4, and 6.

There were significant differences in TP among sites for the four common consumers, but no single site was different than all other sites. For golden perch ($F_{(4,10)} = 23.319$, $p < 0.0001$) 6 of 10 pairwise comparisons were significant. For bony bream ($F_{(5,12)} = 4.643$, $p = 0.014$) only sites 1 and 5 were significantly different. For both carp ($F_{(5,12)} = 18.62$, $p < 0.0001$) and carp gudgeons ($F_{(5,12)} = 17.606$, $p < 0.0001$) 8 of 15 pairwise comparisons were significant (Figure S3).

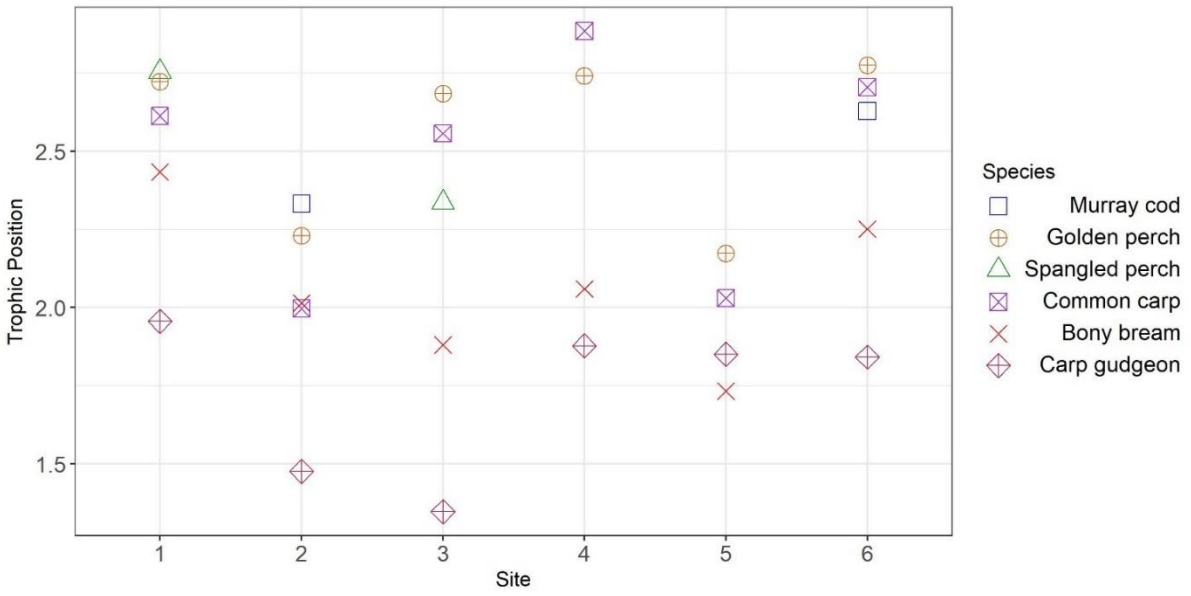

Figure 5-5 Trophic positions calculated based on δ15N values for each species in each site using species-specific trophic enrichment factors. Samples were collected in March 2019 in the Barwon-Darling River.

5.5 Discussion

Flow variability

Flooding and in-channel flows connect floodplains, anabranch channels, and inundate inset 'benches', transferring nutrients into the main channel and waterholes (Bunn et al., 2003; McGinness & Arthur, 2011; Robertson, Burns, & Hillman, 2016). It was expected that antecedent flow patterns would influence nutrient dynamics and availability within sites. We expected to see differences in basal resource quality and quantity reflected in higher trophic levels between sites with varying hydrological character. Broadly, we expected differences between sites with high and low frequency of baseflow and above baseflow events. Sites characterised by low frequency of baseflow events would be more resource restricted and show greater trophic overlap and smaller niche area and FCL. However, there was no pattern between flow frequency and food web structure. For example, site 5 had low hydrological connectivity and had the most condensed isotopic niche space with high species packing and low trophic diversity. It also had the shortest FCL with TPs spanning less than one full trophic level, and a low mean TP. This indicates that there is high omnivory and competition for a limited range of resources (Layman et al., 2007), as was expected in a site with low connectivity. However, sites 3 and 6 also had low hydrological connectivity but had the greatest trophic area, low trophic overlap, higher mean TP and FCLs that spanned at least one full trophic level. This indicates that fish are accessing a greater variety of food resources and there is

reduced competition for the resources available, counter to what was expected in these sites. The lack of a consistent pattern between flow frequency and food web structure suggests that other factors are more important than antecedent flow in structuring food webs in these sites. Samples for this study were collected after an extended dry period that was preceded by an extended period of low flows. All sites had experienced no-flow conditions for eight months prior to sample collection. Thus, it is likely that local conditions had a stronger influence at the time of collection than recent flow patterns.

Effects of drought

Drought affects a range of ecosystem processes and influences community structure both directly and indirectly from nutrient cycling to trophic structure and biotic interactions (Lake, 2003). Rolls, Leigh, and Sheldon (2012) identified links between low flow and riverine ecosystem processes and patterns. They suggest that prolonged periods of low flow may have more significant ecological impacts than frequent short periods of no flow. As the duration of a low-flow period increases, the densities of biota temporarily increase due to reduced volume of habitat and subsequently decrease due to increased competition for resources. Food webs are expected to become compressed and the reduced basal nutrient availability will have corresponding effects up the trophic levels (Rolls et al., 2012).

Stable isotopes are useful in food web studies because they reflect food that is assimilated, not just consumed, and they integrate a longer temporal scale than gut content analysis (Post, 2002b; Winemiller, Akin, & Zeug, 2007). Tissue turnover rates are influenced by a range of factors including body size, which determines the time scale that stable isotope values integrate (Vander Zanden, Clayton, Moody, Solomon, & Weidel, 2015). The allometric equation for ectotherm vertebrates developed by Vander Zanden et al. (2015) indicates that the isotopic half-life based on mean body mass (mean body mass of golden perch, bony bream, and carp = 942 g) is 120 days, with a shorter half-life for carp gudgeons. Thus, isotope values of the fish used in this study may reflect the progression of drought conditions. The date that in-channel flows ceased to connect each waterhole was based on cease-to-flow of nearest gauging station and ranged from mid-June to early August 2018, with a small flow event in November that may have reached site 1, the most upstream site. Assuming shallow waterholes dry faster (i.e. habitat size reduces faster) (Reid, Thoms, Chilcott, & Fitzsimmons, 2017), the effects of drought in the qualitatively smaller habitats should be reflected in isotope values earlier than larger habitats.

As streams dry and habitat size is reduced, so do food chain length (FCL) and trophic area (TA) (McHugh et al. 2015). We expected that sites in this study would reflect increased competition for limited resources with a more compressed food web as drought progresses. However, this pattern was not found. At the time of sampling, site 2 had dried down to a series of disconnected pools and was the smallest site. It had a small trophic area, high species packing, and a short FCL, as was expected for a smaller site. Carp gudgeons in site 2 contributed the most to the size of the trophic area (Figure 3). All other species showed high trophic overlap, indicating that carp gudgeons in this site were utilising a different food resource and there was high competition among the other species. Samples at site 2 were collected from multiple pools, and the pool from which carp gudgeons were collected was shared only with other small-bodied fishes. The lack of predation pressure and competition for resources with larger species such as carp may have contributed to their differentiation from the other species in this site. However, site 6, another small site, was the least similar to site 2 having one of the larger trophic areas and greater trophic diversity with low species packing and a long FCL, counter to what was expected in a small site. Carp gudgeons in site 6 were also contributing to the greater trophic area, but these samples were collected from a pool they shared with all the other species. There was lower competition in this site than in site 2 and golden perch were feeding a full trophic level above carp gudgeons. Site 4, a small site, also had a longer FCL and higher mean TP than larger sites. Despite expectations that these sites would reflect the effects of prolonged drought more than larger sites, there were enough food resources remaining to support the fish populations without high competition. Interestingly, carp gudgeons in these smaller sites had the highest $\delta^{13}C$ values which were significantly different than carp gudgeons in the larger sites, suggesting that they were accessing different basal resources. Assuming shrimp $\delta^{13}C$ signatures reflect primarily benthic basal resources, carp gudgeons in small sites may be relying on benthic resources more than the other species.

In a naturally turbid river, the stable water levels during disconnected periods promotes the establishment of productive benthic algae in narrow photic zones (Bunn, Thoms, et al., 2006), potentially providing enough energy to sustain the biotic community. In the Warrego River, a tributary of the Barwon-Darling, small and shallow waterholes supported more species at higher fish abundances than large and deep waterholes, possibly due to habitat that supports greater potential primary productivity, while the deep waterholes provide important refuge for larger-bodied species (Balcombe et al., 2006). In the present study, a habitat assessment was not conducted. But based on site observations, the potential photic zone that could support benthic algae production was greater at the smaller sites. These habitat characteristics could help explain why there was no pattern of reduced trophic area and short FCL in the small sites in this study. It could also explain why carp

gudgeons appear to be utilising more benthic food resources in smaller sites. However, bony bream, primarily a herbivore and detritivore, did not appear to rely as heavily on benthic resources as carp gudgeons in sites 2 and 6.

Dietary flexibility and generalist feeding strategies may protect food web structure from effects of hydrological disturbance, as was found for macroinvertebrates in seasonally connected wet-dry tropics rivers (Leigh et al., 2010). Consumer's lack of preference for basal sources and their assimilation of multiple sources means that as long as enough basal resources are available, a change in resources due to a change in hydrological connectivity will not change food web structure (Leigh et al., 2010). If this pattern holds for higher trophic levels in a dryland river, the spatial variation in food web structure in these sites is more influenced by local habitat factors than directly by hydrological connectivity and nutrient transfer among sites. Food web metrics we expected to be associated with reduced access to resources and increased competition are not directly related to hydrological connectivity and size of the site. This would explain the lack of a consistent pattern for our site classifications.

Other studies have found that floodplain carbon subsidies are important for maintaining fish populations in floodplain rivers (Burford, Cook, Fellows, Balcombe, & Bunn, 2008; Jardine et al., 2012; Warfe et al., 2013). During times when the Barwon-Darling River is flowing, the spatial habitat boundaries for the species used in this study is large. Bony bream, golden perch, spangled perch, and Murray cod are capable of large-scale movement, and their high genetic connectedness throughout the river basin and observations at fish passages suggest that they disperse across these habitat patches (Chapter 6; Attard et al., 2018; Bostock, Adams, Laurenson, & Austin, 2006; Harrisson et al., 2017). The influence of floods and high flows on trophic interactions could not be directly assessed in this study. Classifications of hydrological connectivity patterns were derived based on the previous five years and as a result are primarily assessing the recent influence of longitudinal connectivity due to overall low flows. The influence of local habitat versus connectivity on trophic interactions likely varies with flow conditions, but the relative importance of each in the long-term persistence of fish in this river is an important factor to assess.

Spatial distribution of waterholes

Location of waterholes in relation to weirs and other waterholes may also play a role nutrient dynamics and biotic interactions. Interconnectedness of waterholes influences fish, algal, and macroinvertebrate communities, with waterholes that are physically closer to each other being more closely related in faunal composition (Arthington et al., 2005; Marshall, Sheldon, Thoms, &

Choy, 2006; McGregor, Marshall, & Thoms, 2006). Relatedness in faunal composition among waterholes may also extend to trophic interactions of fish consumers. We expected that increasing isolation of sites would limit access to resources from other habitat patches, resulting in decreased trophic area, increased trophic overlap, and lower FCL and mean TP due to higher rates of omnivory.

Although not measured directly, based on findings of Pearson et al. (2020), we assumed that sites within weir pool influence are more connected to nearby waterholes than sites outside of weir pool influence. The large sites in this study were within a weir pool influence and were expected to have greater access to resources. Site 3 had the largest trophic niche area, low trophic overlap, and the largest TP range, indicating that there was low competition for resources as expected. However, in terms of community metrics, site 3 was most similar to site 6, a small and isolated site. Fishes in both sites occupied a large trophic area and had low trophic overlap. At the same time, site 5 reflected high competition for resources, counter to what was expected. There was no consistent pattern based on our assumed spatial distribution of waterholes. Most likely these sites are more strongly influenced by local conditions due to the prolonged drought conditions than potential connectedness to other habitats.

Influence of omnivory in food web studies

Widespread and common species within the Murray-Darling Basin have adapted to the variable hydrology and possess traits that convey resistance and resilience to droughts (Baumgartner, Wooden, Conallin, Robinson, & Thiem, 2017; McNeil, Gehrig, & Sharpe, 2013). Hydrologically variable environments require opportunism and dietary flexibility (Behn & Baxter, 2019; Pusey et al., 2010), and omnivory is a response to less-predictable food resource availability (Blanchette et al., 2014). Omnivory and generalist feeding habits allow consumers to use resources more efficiently (Sternberg, Balcombe, Marshall, & Lobegeiger, 2008) and simplicity of trophic structure and high rates of omnivory are expected in highly variable rivers (Douglas et al., 2005; Poff & Allan, 1995). Species used in this study have generalist feeding behaviour and the ability to shift their diet based on resource availability (Reid et al., 2012; Sternberg et al., 2008). For example, both carp and carp gudgeons show a preference for invertebrates but supplement their diet with detritus when resources are limited (Balcombe & Humphries, 2006; Khan, 2003). Similarly, golden perch can switch between high energy prey and low energy prey based on rates of primary productivity (Sternberg et al. 2008).

It was predicted that omnivory would be high at all sites, but that smaller and more hydrologically isolated sites would reflect greater competition for resources with increased omnivory. We expected

shorter FCL and a smaller range of TPs. Sites 1 and 5 had contrasting hydrological characteristics but they had the smallest TP ranges. Golden perch, a carnivore, was feeding less than one trophic position above bony bream, primarily a herbivore and detritivore, indicating that golden perch was feeding at a lower trophic position. These two sites spend a similar proportion of time in cease-to-flow conditions, but site 1 experiences more frequent baseflow and above events and shorter dry spells. These were both larger sites and were expected to have less competition for resources and less omnivory than smaller sites. Site 3 was hydrological isolated like site 5, but had the largest TP range, and low trophic overlap, indicating lower rates of omnivory at site 3. There was no consistent pattern between site classifications and rates of omnivory. This study was restricted to only four species, but they represent a range from herbivores to carnivores. Golden perch and carp were very similar in TP at all sites and at several sites were less than half a trophic position away from bony bream, suggesting that golden perch were feeding relatively low on the food chain at all sites. Macrocrustaceans are an important food resource for golden perch (Baumgartner, 2007) and the use of *M. australiense* as the baseline for calculating TP likely contributed to their low TP at all sites.

Study limitations

Nutritional status of organisms may influence values of $\delta^{13}C$ and $\delta^{15}N$. Studies in fish have indicated that nutritional stress may influence $\delta^{13}C$ due to lipid depletion during periods of fasting or starvation (Gaye-Siessegger, Focken, Abel, & Becker, 2003; Gaye-Siessegger, Focken, Muetzel, Abel, & Becker, 2004). However, larger reviews have found that trends in the response of $\delta^{13}C$ values are inconsistent (Hatch, 2012; Hertz, Trudel, Cox, & Mazumder, 2015). The response of $\delta^{15}N$ values on the other hand are more consistent. During periods of severe fasting or starvation, tissues become enriched in ^{15}N when animals are catabolizing their own tissue, with increasing enrichment as duration of nutritional stress increases (Del Rio & Wolf, 2005; Vidal, González-Bergonzoni, & Naya, 2018). However, several studies have found that the effects are varied and tend to be small in comparison to other factors influencing $\delta^{15}N$ values (Hatch, 2012). A meta-analysis found that the effect of nutritional restriction on $\delta^{15}N$ is an average of 0.5‰ increase, but varies by tissue type (Hertz et al., 2015). While it is likely that all fishes in this study were experiencing some level of nutritional stress due to the prolonged period of no-flow prior to collection of samples, the high rate of omnivory and generalist feeding likely had a greater influence than nutritional stress on TP at the scale of food web analysis used in this study.

Identifying primary sources of carbon supporting food webs in isolated waterholes in the Barwon-Darling River was outside the scope of this study. Primary consumers were chosen to represent basal

resources and make inferences about the use of benthic and pelagic carbon sources at these sites. The consumers, freshwater shrimp and mussels, used in this study to represent baseline resources are not obligate primary consumers, which may account for ^{15}N enrichment compared to fishes. The freshwater shrimp *Macrobrachium australiense* is omnivorous (Burns & Walker, 2000a) and has been shown to reflect benthic algal carbon signatures (Bunn et al., 2003). However, it will feed on most of the potential food resources, including invertebrate larvae, and may also be scavengers (Burns & Walker, 2000b). Mussels had higher $\delta^{15}N$ values than many of the fishes, with the sample from site 2 having a similar signature to Murray cod, a carnivore. There are a few potential explanations for this. Mussels may not feed solely as primary consumers and may utilise a different resource pool, such as bacteria or phytoplankton, than fishes (Vaughn, Nichols, & Spooner, 2008). Freshwater mussels are long-lived sedentary filter-feeders that integrate long-term nutrient conditions (Cabana & Rasmussen, 1996; Gustafson, Showers, Kwak, Levine, & Stoskopf, 2007) and larger (i.e. older) mussels, which were used in this study, are able to consume organisms higher in the food chain resulting in higher $\delta^{15}N$ values (Howard, Cuffey, & Solomon, 2005). While periods of physiological stress can also result in higher $\delta^{15}N$ values in mussels due to catabolism (Patterson & Carmichael, 2018), similar to effects in fish, this influence is small relative to trophic enrichment.

5.6 Conclusion

It was expected that greater hydrological connectivity and larger waterhole size would result in greater access to resources and less competition. However, there were no consistent patterns identified between food web metrics and hydrological classifications of sites. This study was conducted during a drought and isotope values were more likely reflecting local site conditions in an extended no-flow period than the influence of antecedent hydrology. Longer-term stable flow conditions and habitat characteristics of smaller, less connected sites may have promoted primary productivity which sustains the higher trophic levels.

A more thorough assessment of food web structure is necessary to establish a baseline to measure the effect of water resource development. This study identified that there is spatial variation in food web structure within the river, but that it is not directly related to recent hydrological character. The physical habitat characteristics of each site are likely a stronger influence on food web structure during prolonged drought. Studying temporal changes in food web structure during and after a flow event, as well as assessing habitat characteristics and identifying sources of primary production, are necessary to resolve the factors influencing food web structure.

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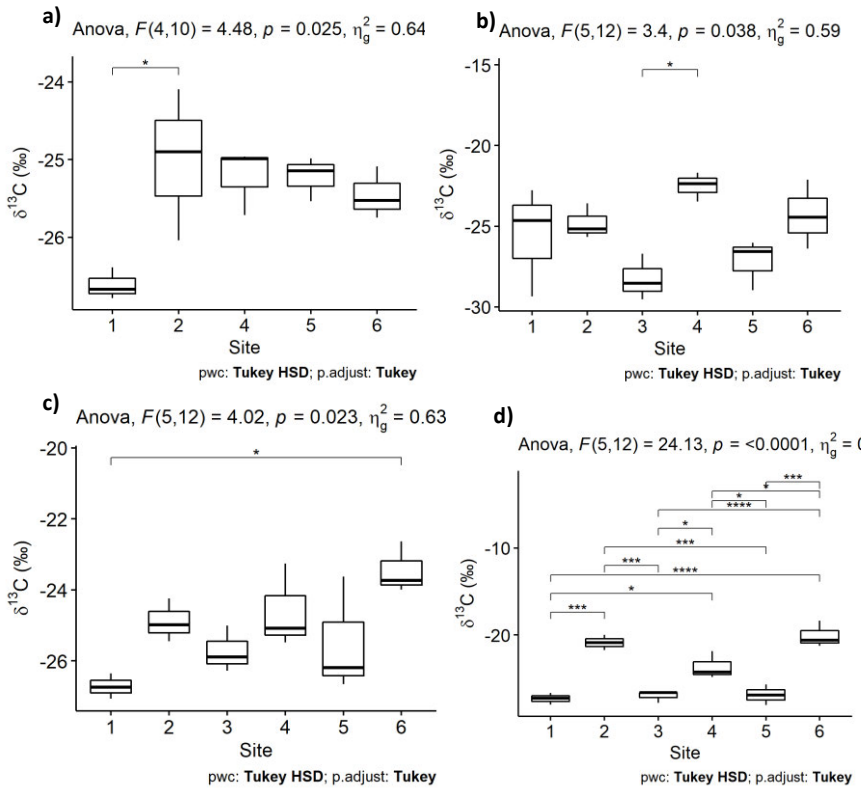

Figure 5-S1 $\delta^{13}C$ ANOVA pairwise comparisons assessed between sites for each species with Tukey's HSD test (* is significance level of 0.05). Plot a) golden perch; plot b) bony bream; plot c) common carp; plot d) carp gudgeons. Samples collected in March 2019 in the Barwon-Darling River.

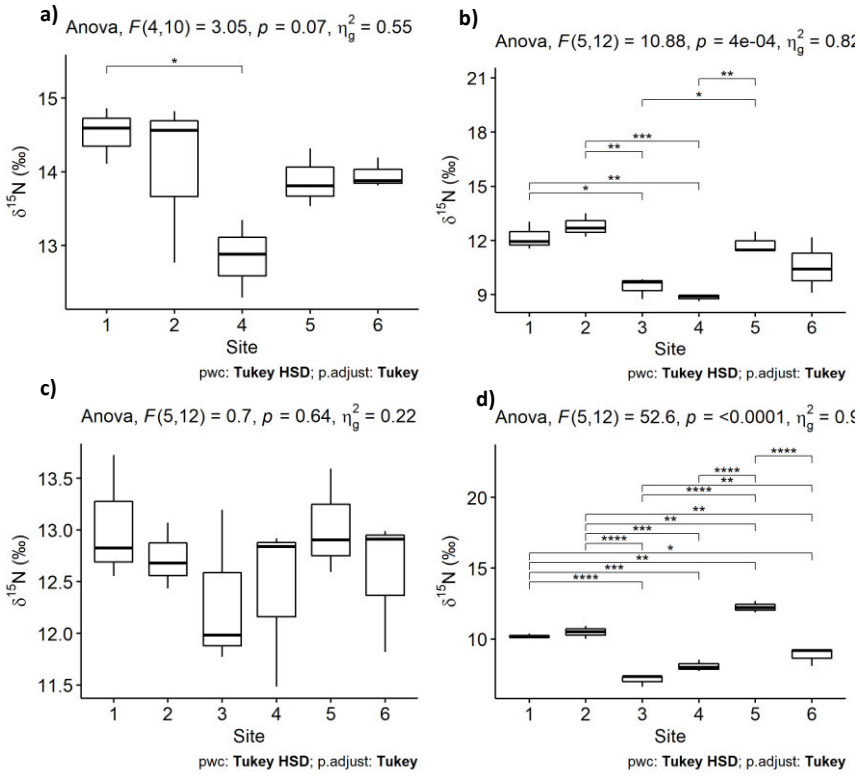

Figure 5-S2 $\delta^{15}N$ ANOVA pairwise comparisons assessed between sites for each species with Tukey's HSD test (* is significance level of 0.05). Plot a) golden perch; plot b) bony bream; plot c) common carp; plot d) carp gudgeons. Samples collected in March 2019 in the Barwon-Darling River.

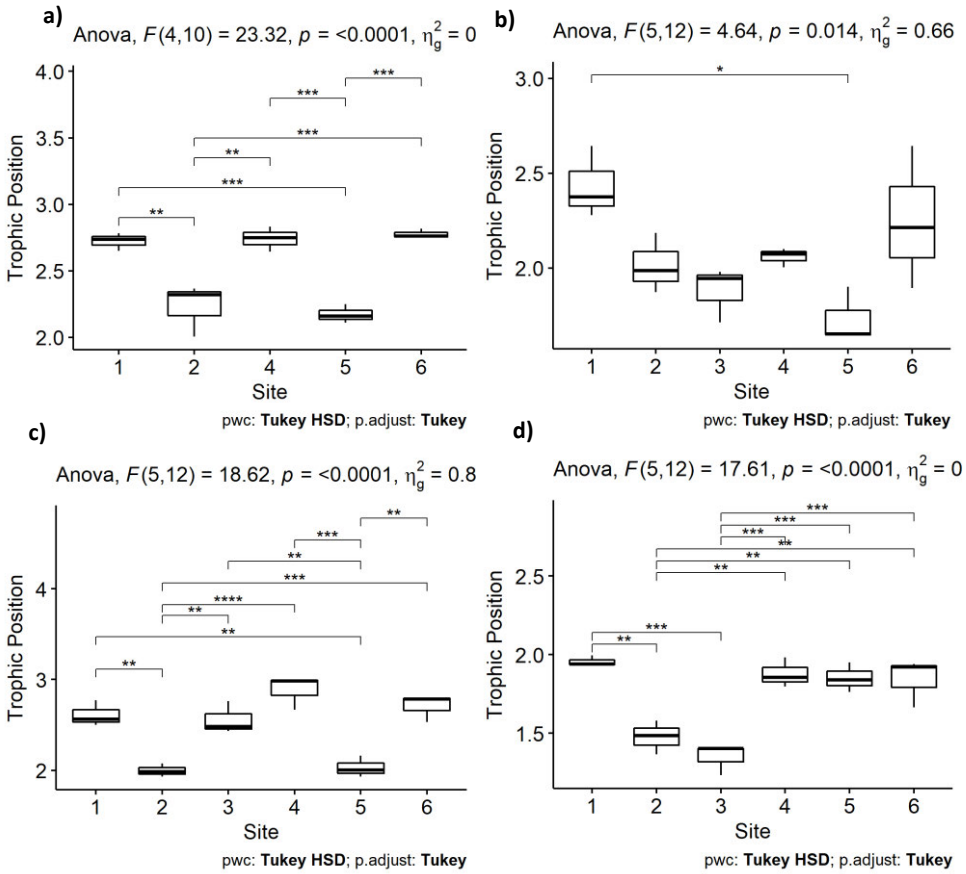

Figure 5-S3 Trophic positions ANOVA pairwise comparisons assessed between sites for each species with Tukey's HSD test (* is significance level of 0.05). Plot a) golden perch; plot b) bony bream; plot c) common carp; plot d) carp gudgeons. Samples collected in March 2019 in the Barwon-Darling River.

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Candidate

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Principal Supervisor

23/10/2020
Date

Population structure of Bony Bream (*Nematalosa erebi*) in the Barwon-Darling River

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6.1 Abstract

Fragmentation of river habitats as a result of water resource development restricts movements of organisms and threatens the persistence of species. Dryland rivers have highly variable flow regimes with natural periods of disconnection. However, altered flow regimes and physical barriers have increased this fragmentation. Functional connectivity within the Barwon-Darling River in Eastern Australia was assessed based on genetic structure of bony bream, *Nematalosa erebi,* a common and widespread species. Samples were collected from eight sites along the length of the main river channel and two remote sites, one from a tributary river and one from near the mouth of the Murray River. Analysis of a single nucleotide polymorphisms (SNP) dataset was used to assess if genetic structure is related to anthropogenic fragmentation. There was differentiation among sites, but there was no positive correlation between genetic structure and geographic or resistance distance between sites. Anthropogenic fragmentation of the river has occurred relatively recently and detecting any genetic response likely requires several more generations. Similar to other species in extinction prone environments, *N. erebi* may rely on metapopulation dynamics to persist in this highly variable river.

6.2 Introduction

Worldwide, habitat loss and fragmentation threaten the persistence of species (Fischer & Lindenmayer, 2007). Freshwater ecosystems in particular have experienced a decline in biodiversity, with more than 20 percent of fish species threatened, endangered, or become extinct in the recent decades (Revenga, Brunner, Henninger, Kassem, & Payne, 2000). In river systems, habitat fragmentation as a result of water resource development has changed the structure and function of riverine ecosystems (Benke, 1990; Liermann, Nilsson, Robertson, & Ng, 2012; Nilsson, Reidy, Dynesius, & Revenga, 2005). The construction of dams, weirs, levees, and other hydrological alteration fragments habitats, restricts movements, and alters ecosystem processes (Fuller, Doyle, & Strayer, 2015). The dendritic nature of rivers makes them particularly vulnerable to anthropogenic fragmentation (Fagan, 2002). The riverine network configuration naturally constrains the connectivity of obligate freshwater organisms (Campbell Grant, Lowe, & Fagan, 2007), and anthropogenic fragmentation further restricts their movement. Within Australia, projected reductions in surface water availability due to climate change (CSIRO, 2008; Morrongiello et al., 2011) further magnify the threats posed by water resource development (Balcombe et al., 2011). In the face of this unprecedented environmental change, understanding a species' capacity to persist is imperative.

Landscape genetics provides a framework for understanding the impact of fragmentation on genetic connectivity. Landscape connectivity can be defined as "the degree to which the landscape facilitates or impedes movement among resources patches" (Taylor, Fahrig, Henein, & Merriam, 1993). Structural connectivity of a landscape is a measure of habitat contiguity and can be assessed based on landscape structure. Functional connectivity, on the other hand, is a measure of an organism's behavioural response to various elements within a landscape (Tischendorf & Fahrig, 2000). Structural connectivity is relatively easy to measure but does not necessarily reflect functional connectivity. In rivers, hydrological connectivity (i.e. structural connectivity) alone is a poor predictor of realised connectivity (Hughes, Huey, & Schmidt, 2013). Genetic markers provide a tool for assessing functional connectivity given that connected populations will share alleles and allele frequencies, and populations that are disconnected will differ in allele frequencies (Slatkin, 1993). Landscape genetics considers both structural and functional connectivity by incorporating spatial data with population genetics (Grummer et al., 2019; Lean, Hammer, Unmack, Adams, & Beheregaray, 2017; Manel, Schwartz, Luikart, & Taberlet, 2003). It combines concepts from landscape ecology with genetic analysis to examine how landscape features influence micro-evolutionary processes at multiple spatial and temporal scales (Anderson et al., 2010; Manel et al., 2003), providing a measure of functional connectivity (Manel & Holderegger, 2013) across structurally connected landscape elements (Manel et al., 2003).

Dryland rivers

Dryland rivers in Australia experience intermittent and unpredictable flows (Powell, 2009; Walker, Sheldon, & Puckridge, 1995), exhibiting some of the highest levels of flow variability under natural flow conditions (Puckridge, Sheldon, Walker, & Boulton, 1998). Natural fragmentation, or loss of hydrological connectivity, is a function of the flow regime and generates a network of variably connected channels and waterholes in an otherwise dry river bed (Poff et al., 1997; Puckridge, Walker, & Costelloe, 2000; Sheldon & Fellows, 2010). Variable connectivity is an integral part of ecosystem function within dryland rivers (Sheldon, Boulton, & Puckridge, 2002; Walker et al., 1995), and many riverine organisms have life history traits that are adapted to cope with and use the natural variability of lotic systems to their advantage (Lytle & Poff, 2004). Organisms inhabiting variable environments, such as temporary rivers, have responses to habitat contraction that promote their survival such as a period of dormancy or migration to refugia (Larned, Datry, Arscott, & Tockner, 2010). In dryland rivers refugial waterholes act as important habitat during periods of low or no flow (Arthington, Balcombe, Wilson, Thoms, & Marshall, 2005). Despite the loss of structural connectivity during these periods, functional connectivity is retained because under natural conditions waterholes reconnect within many species' lifetimes (Sheldon et al., 2010).

The reduction or cessation of river flows is expected and linked to climate, but water control structures and water abstraction for human use creates 'artificial droughts' (Boulton, 2003), which extend or result in periods of unnaturally low or no flow. Physical barriers further artificially fragment the river habitat, restricting movement of organisms among waterholes during periods of low flow (Baumgartner, Zampatti, Jones, Stuart, & Mallen-Cooper, 2014). Additionally, this water resource development threatens the persistence of refugial habitat due to increased sedimentation in waterholes (Pearson, Reid, Miller, & Ryder, 2020; Reid, Thoms, Chilcott, & Fitzsimmons, 2017).

The Barwon-Darling River, within the Murray Darling Basin, is a dryland river flowing through arid to semi-arid landscape for much of its length in New South Wales, Australia. It has a catchment area of 699,000 km^2, 60% of which is less than 300 m above sea level, and a main channel length of 2,700 km (Webb Mckeown, 2007). The Barwon-Darling River and its major tributaries are low gradient rivers exhibiting seasonality in flows, existing for most of the time in a low-flow stage with high flows occurring during summer and autumn months (Thoms, Sheldon, & Crabb, 2004). The river is classified as having unpredictable summer dominated flows, which are highly intermittent (Kennard et al., 2010). The system has undergone extensive modification since the 1960s in the form of dams, weirs, and water extraction. Prior to regulation, the river flowed 92% of the time and periods of no flow were short (Mallen-Cooper & Zampatti, 2020). Water resource development has resulted in an increase in the length of cease to flow periods, and a decrease in frequency and number of small flow pulses (MDBA, 2016). The magnitude of near-annual flow pulses has been reduced by over 90% and the percentage of time the river exists in lentic condition has doubled (Mallen-Cooper & Zampatti, 2020). Flows during dry periods are largely maintained through the delivery of environmental water (Sharpe & Stuart, 2018).

There are no large dams on the main channel, but there are a number of low weirs. The low grade of the river means that weirs limit dispersal at low to moderate flows, restricting movement opportunities for fish to infrequent and irregular high flows when water overtop the weirs (Baumgartner et al., 2014; Gehrke, Brown, Schiller, Moffatt, & Bruce, 1995). Water regulation in the river has contributed to reduced abundance and species diversity of native fish (Gehrke, 1997; Gehrke et al., 1995). It is estimated that native fish populations exist at about 10% of their pre-European settlement levels (MDBA, 2004).

Species overview

Nematalosa erebi, bony bream, is one of the most widely distributed freshwater fish species in Australia and is frequently found in high abundance. *N. erebi* mature at 12 to 24 months, with a lifespan of up to five years, and are highly fecund (Pusey, Kennard, & Arthington, 2004). They exhibit

opportunistic spawning behaviour, with evidence of recruitment during no-flow periods (Balcombe et al., 2006). *N. erebi* primarily consume zooplankton as juveniles and switch to detritus and algae as adults (Pusey et al., 2020). They play an important role in nutrient transfer and represent a vital food source for higher-order consumers (Pusey et al., 2004). Adult fish are commonly 120 to 300 mm but can reach a maximum size of 470 mm (Lintermans, 2007). Their morphology suggests they are capable of largescale movement, though dispersal has only been inferred from observations at fishways (Barrett & Mallen-Cooper, 2006) and limited acoustic tagging (Marshall et al., 2016). These traits indicate that they are likely to be minimally impacted by climate change scenarios (Balcombe et al., 2011), and their movement ability and high fecundity should provide opportunities for gene flow. However, Gehrke (1997) found reduced abundance in regulated reaches in the Murray-Darling Basin. Hughes and Hillyer (2006) investigated historical connectivity of *N. erebi* using mitochondrial DNA and allozymes. Within two northern tributary catchments of the Murray-Darling Basin, they found significant genetic differentiation between catchments and some evidence of differentiation among waterholes, as well as a pattern of isolation-by-distance within these tributaries. A phylogeographic study across the distribution range of *N. erebi* indicated that populations in the Murray-Darling Basin are closely related (Bostock, 2014).

Aims

In this study, we aim to investigate the genetic structure of *N. erebi* using genomic analysis to better understand the species' potential to persist under future environmental change. Specifically, we seek to determine if fine-scale population structure reflects reduced structural connectivity within the Barwon-Darling River and if existing population structure is associated with habitat fragmentation due to weirs. Results will further our understanding of the ecology of *N. erebi*, an ecologically important, but understudied, species.

6.3 Methods

Specimen collection

Samples of *N. erebi* were collected from the main channel of the Barwon-Darling River in New South Wales, Australia. Sampling was done in September 2018 and March 2019. Samples were collected opportunistically during fish community surveys conducted by NSW DPI Fisheries Research using standard boat and backpack electrofishing methods (MDBC, 2008). Attempts were made to standardise the size class of fish from which samples were taken. However, due to low catch rates

samples were collected when fish were over 150 mm fork length, or from the largest individuals when all were below 150 mm. Up to 10 individuals per site were sampled. Small fin clips were taken from the distal portion of the upper caudal fin, preserved in RNALater or 95% ethanol, and stored at -20°C. Samples were taken from live fish which were released at the site of collection. The collection of animals used in this study complied with University of New England's animal research authority AEC 18-051 and NSW DPI ACEC Permit No. 98/14.

A total of 74 specimens to be genotyped were selected from five sites where at least 10 individuals were captured, and an additional three sites with fewer than 10 individuals (Figure 1). These eight sites are distributed within the main channel of the river and are considered to be main channel populations for this study. An additional 10 individuals from the Warrego River (WR), an intermittent river and tributary of the Darling River, and 10 individuals from the Murray River (MR), downstream of the confluence with Darling River, were provided for comparison. The samples from WR and MR were juveniles collected in 2015 and 2014, respectively. The sites from these two rivers are considered to be remote populations.

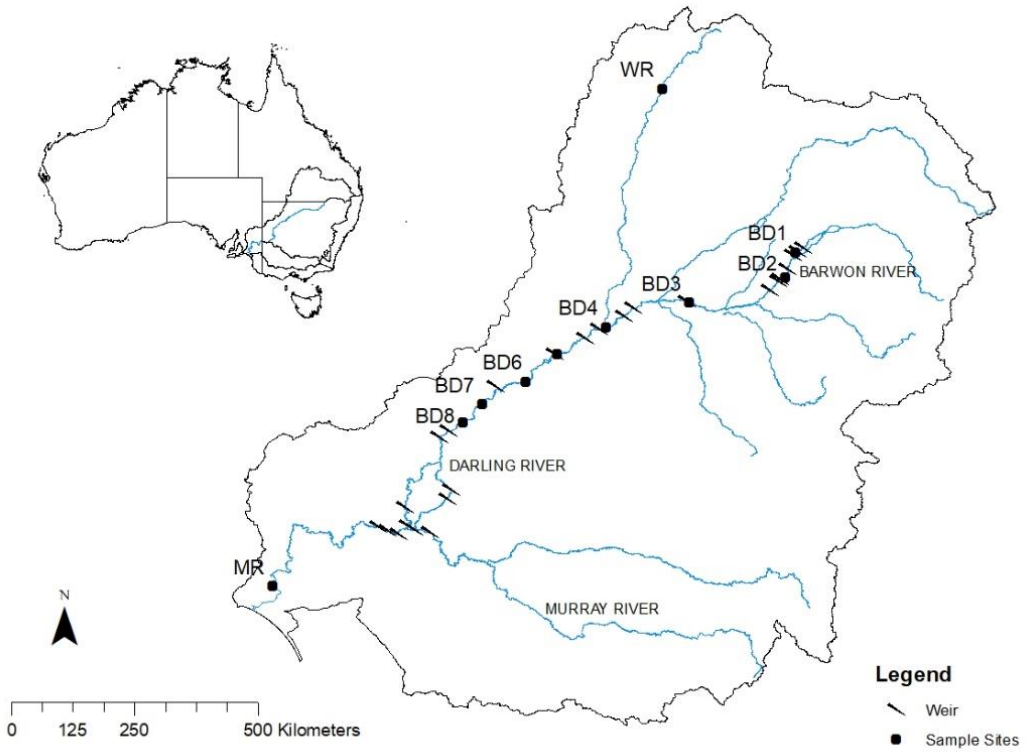

Figure 6-1 Location of sample collection sites within the Murray-Darling Basin, Australia.

DNA extraction and sequencing

DNA was extracted, sequenced, and informative single nucleotide polymorphism (SNP) markers were identified by Diversity Arrays Technologies (DArT Pty Ltd, https://www.diversityarrays.com). Genotyping-by-sequencing was performed by DArTseq, which uses restriction enzymes for complexity reduction and next-generation sequencing platforms as described by Kilian et al. (2012).
Data filtering

Filtering of SNPs was facilitated with the dartR package v. 1.5.5 (Gruber, Unmack, Berry, & Georges, 2018) in R version 3.6.1 (R Core Team, 2018). Only SNPs with 100% repeatability were retained. Data were filtered for call rate at 95% for both individuals and loci, retaining SNPs for which there is less than 5% missing data. Secondary SNPs, where they occurred in a single sequenced tag, were removed, retaining the SNP that had the highest information content (avgPIC). SNPs were filtered to remove loci with minor allele frequency <0.05. Loci were filtered for deviations from Hardy-Weinberg equilibrium with all sites pooled using Fisher's Exact Test at a significance of 0.001 with Bonferroni correction.

To ensure a neutral set of SNP markers when assessing genetic connectivity among populations (Luikart, England, Tallmon, Jordan, & Taberlet, 2003), data were filtered for loci not conforming to expectations of neutral distribution using two methods. Loci potentially under selection were detected in BAYESCAN v. 2.1 (Foll & Gaggiotti, 2008) which uses a Bayesian method to estimate population-specific F_{ST} coefficients (Beaumont & Balding, 2004) and determines a cut-off based on the posterior distribution. BAYESCAN was run with a burn-in of 50,000 and 100,000 iterations with prior odds set at 10. File conversion for BAYESCAN format was done in DartR. Outlier loci were also detected with OutFLANK v.0.2, which derives the null distribution of genetic differentiation for neutral loci by removing loci in the top and bottom 5% of the distribution (Whitlock, Lotterhos, & Editor: Judith, 2015). OutFLANK was run with default settings and a false discovery rate of 5%. Files for OutFLANK format were converted using genomic converter in the Radiator package (Gosselin, Lamothe, Devloo-Delva, & Grewe, 2020).

Population genetic variation

Sampling sites were considered separate populations for downstream analysis. Genetic variation and structure were assessed using the full filtered SNP dataset. Relative genetic diversity was measured as the observed heterozygosity (H_O) within each population. Observed and expected heterozygosity (H_E), and the inbreeding coefficient (F_{IS}), based on allele frequencies were obtained using DartR v. 1.5.5 (Gruber et al., 2018).

Population differentiation and genetic structure

Population structure was inferred using a discriminant analysis of principal components (DAPC) (Jombart, Devillard, & Balloux, 2010) in adegenet v 2.1.1 (Jombart, 2008). DAPC partitions the variance into within-group and between-group components, and maximizes the discrimination between groups, detecting population clustering even in the presence of weak differentiation, with no assumption about population models (Jombart et al., 2010). The number of principal components (PCs) was chosen to optimize the α-score (optimal number:28), this results in a loss of some power of discrimination but avoids overfitting the model. Successive *K*-means clustering (*find.clusters* function) identifies population clusters that can help explain the variation in the data without prior population groupings. To test if sample sites reflect population clusters, ten iterations of *K*-means clustering were run for each value of *K* 20. Bayesian Information Criterion (BIC) was used to choose optimal number of clusters. Pairwise population genetic differentiation using Wright's fixation index (F_{ST}) was measured in DartR v. 1.5.5 (Gruber et al., 2018), using the method proposed by Weir and Cockerham (1984), with significance assessed over 10,000 bootstraps.

Isolation by distance and Isolation by resistance

To investigate the influence of geographic and anthropogenic factors on spatial genetic differentiation, we tested for a pattern of both isolation-by-distance (IBD) and isolation-by-resistance (IBR). IBD (Wright, 1943) predicts a positive relationship between genetic distance and geographic distance. Due to limited dispersal, individuals that are close to each other are more likely to be genetically similar than individuals that are farther apart. IBR predicts a positive relationship between resistance distance and genetic differentiation, accounting for heterogeneity in distribution and dispersal of species (McRae, 2006). IBR uses resistance surfaces to understand how landscape characteristics influence functional connectivity, by identifying features that facilitate or impede genetic connectivity (Spear, Balkenhol, Fortin, McRae, & Scribner, 2010).

The role of spatial distance in shaping landscape genetic pattern was assessed using a Mantel test (Mantel, 1967) and Mantel correlogram (Oden & Sokal, 1986) to test for spatial autocorrelation. IBD was evaluated using pairwise genetic distances, linearized F_{ST} values [F_{ST} /1- F_{ST}] and non-transformed geographic distances (km) due to linear nature of river habitat (Rousset, 1997). Mantel tests and correlograms were tested with all groups and with remote groups removed. Euclidean and waterway distance were both used for Mantel tests and Mantel correlograms. Due to the dendritic spatial structure of rivers, waterway distance is more representative of true geographic distance between sites than Euclidean distance. Pairwise waterway distances between sampling locations were measured in the R package Riverdist v. 0.15.0 (Tyers, 2017) using spatial data obtained from

the Australian Hydrological Geospatial Fabric v.2.1.1 (Bureau of Meteorology, 2014). Mantel tests were based on Pearson's product-momentum correlation with 10,000 permutations. For correlograms, Sturges equation (Sturges, 1926) was used to set the number of distance classes at seven, and tests used Pearson's correlation with 10,000 permutations and significance (α=0.05) corrected for multiple testing with Holm correction. Calculations were done in R package vegan version 2.5.6 (Oksanen et al., 2019).

To test for IBR, a resistance matrix was developed based on attributes from the National Environmental Stream Attribute Database v1.1 (Stein, Hutchison, & Stein, 2012) an attribute table linked by stream segment number to the Australian Hydrological Geospatial Fabric v.2.1.1 (Bureau of Meteorology, 2014). Stein, Stein, and Nix (2002) characterized the extent of anthropogenic river disturbance based on a set of catchment and flow regime indicators. Stream segments were scored according to the level of potential impact, and disturbance indices were derived. Attributes used to create resistance matrices were: the impoundment factor (IMF), computed based on in-stream structures; the flow diversion factor (FDF), a measure of impact of alterations to the flow regime due to diversion of enhancement of discharge; the section flow regime disturbance index (SFRDI), a weighted sum of IMF, FDF; and a levee bank factor. For details see Stein et al. (2002). Values were transformed to range from one (no disturbance) to 100 (most disturbance). Rasters based on index values were created in ArcMap v10.4.1.

Resistance values were calculated using Circuitscape (McRae, Shah, & Mohapatra, 2013) which uses circuit theory to calculate the resistance cost for movement between two points and produces pairwise resistance distances. Due to the dendritic nature of rivers, and the fact that fish movement among sites is restricted to the river channel, raster cells outside the river channel were coded as missing data. Resistance distance comparisons were made between the three different landscape matrices, following the path of the river. Mantel tests were used to test if genetic distance is correlated with landscape resistance from barriers (IMF), flow alteration (FDF), or a combination of both (SFRDI). Partial Mantel tests were used to evaluate if any correlation between genetic distance and resistance distance remained significant when partialling out the influence of geographic distance.

Table 6-1 Genetic variation of *Nematalosa erebi* in the Barwon-Darling River based on 696 SNPs, and additional information for each sampling location. N sample size; H_O mean observed heterozygosity; H_e, mean unbiased expected heterozygosity; F_{IS}, inbreeding coefficient. Values in parentheses are standard deviations.

Site	Sample Date	*n*	H_o	H_e	F_{IS}	F_{IS} CI
BD1	Apr-19	10	0.205 (0.157)	0.206 (0.148)	0.028	(0.030 - 0.086)
BD2	Mar-19	10	0.216 (0.165)	0.231 (0.145)	0.073	(0.090 - 0.142)
BD3	Mar-19	10	0.217 (0.178)	0.221 (0.153)	0.054	(0.046 - 0.100)
BD4	Mar-19	10	0.211 (0.175)	0.213 (0.154)	0.05	(0.033 - 0.089)
BD5	Mar-19	10	0.231 (0.176)	0.228 (0.149)	0.033	(0.016 - 0.068)
BD6	Sep-18	7	0.252 (0.216)	0.233 (0.162)	0.004	(-0.036 - 0.040)
BD7	09/2018; 03/2019	9	0.221 (0.207)	0.216 (0.162)	0.045	(0.002 - 0.081)
BD8	Sep-18	8	0.227 (0.192)	0.222 (0.161)	0.035	(0.016 - 0.085)
MR	2014	10	0.241 (0.200)	0.226 (0.157)	0	(-0.048 - 0.020)
WR	2015	10	0.217 (0.163)	0.217 (0.141)	0.033	(0.022 - 0.082)
Mean by Pop			0.2238 (0.1311)	0.2213 (0.1532)	0.0355	
Overall			0.224	0.234	0.046	

6.4 Results

SNP Filtering

The unfiltered dataset comprised 94 genotypes with 16,796 polymorphic SNP loci. After stringent filtering on repeatability (100%), call rate (95%), removal of secondaries the dataset was reduced to 4,960 SNP loci. Removing loci with MAF <0.05 further reduced the dataset to 703 SNP loci. Filtering for deviations from HWE removed seven loci, resulting in a final dataset of 696 SNPs. No individuals were removed. There were no outlier loci detected in either Bayescan or OutFLANK. Population Genetic Diversity

Observed heterozygosity (H_o) among populations ranged from 0.205 to 0.252 and were generally similar to expected heterozygosity (H_e) values which ranged from 0.206 to 0.233. Overall H_o of 0.224 was slightly lower than overall H_e of 0.234. Inbreeding coefficient (F_{IS}) ranged from 0 to 0.073, with an overall F_{IS} of 0.046 (Table 1).

Population differentiation and genetic structure

The DAPC plot showed a separate cluster for the MR population and overlapping clusters for remaining populations (Figure 2). The remote populations also showed a high rate of successful reassignment to their original populations (MR 1.0; WR 0.8). Main channel population reassignment values were between 0.2 and 0.6 suggesting main channel populations may be admixed. *K*-means clustering identified two or three as being the most likely number of clusters. Cumulative variance increased almost linearly with the number of PCs and 90 PCs were retained for analysis to explain more than 90% of total variance. The range of BIC values for one to three clusters was small, with a difference of only 1.26 between K=1 and K=3, and 0.126 between k=2 and k=3. For both two and three clusters, MR was the only pre-defined group to be reassigned together in the cluster analysis, suggesting that these individuals are more similar to each other than other populations. However, the MR group did not cluster separately as a population, with individuals from other locations clustering with the MR group. Pairwise genetic differentiation analysis indicated no to low levels of genetic differentiation (Hartl, Clark, & Clark, 1997), with F_{ST} values ranging from 0 to 0.133 (Table 2). Allele frequencies were significantly different in twenty-seven out of the 45 pairwise comparisons ($p < .01$). The highest significant F_{ST} was 0.133 between the two remote populations of MR and WR, which are among the sites with the greatest distance between them (2,442 km river distance). No single site was significantly differentiated from all other sites, and there was no evident relationship between F_{ST} values and geographic distance. For example, BD1, the most upstream site, was more differentiated from site BD2, the next downstream site, than it was from BD3, BD4, and BD5, while again being significantly differentiated from site BD7 but not BD8 (Table 2).

The Mantel correlograms for both Euclidean and waterway distance had similar patterns and indicated that there is significant but weak positive spatial correlation for the first two distance classes from 0 to 600 km (waterway distance), and significant negative spatial correlation for the last distance class of 1,999 to 2,348 km (waterway distance), with no significant correlation detected for mid-distance classes (Figure 3a). This indicates that populations within 600 km are more similar to each other than expected at random and more dissimilar at the farthest distances sampled. There is some spatial autocorrelation, and the MR group is driving a pattern of IBD. Mantel tests also indicated a significant correlation between genetic and geographic distances for both Euclidean

distance (r = 0.756, p < 0.001) and waterway distance (r = 0.793, p = .002). When remote populations (MR, WR) were removed, there was no significant indication of IBD based on either the correlogram or Mantel test (r = 0.137, p = .198) (Figure 3b).

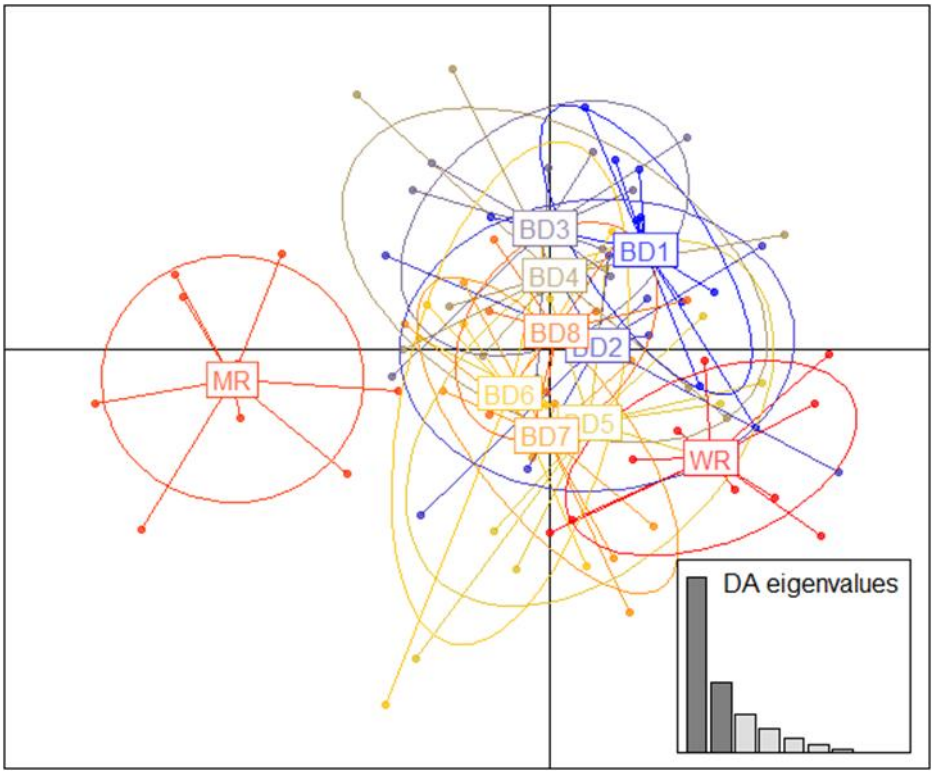

Figure 6-2 Discriminant Analysis of Principal Components (DAPC) for *Nematalosa erebi* from sites within the Barwon-Darling River (BD), the Warrego River (WR) and the Murray River (MR), based on 696 SNPs.

There was no indication of IBR for any of the resistance matrices. Mantel tests did not indicate a strong correlation between genetic distance and resistance distance for IMF (r = 0.403, p = .057), FDF (r = 0.250, p = .231), or SFRDI (r = 0.334, p =.105). Partial Mantel tests between genetic and resistance distances while controlling for the effects of river distance suggested a non-significant negative correlation for all three matrices (IMF: r = -0.518, p =0.998; FDF: r = -0.625, p = .999; SFRDI: r = -0.586, p = .999).

Table 6-2 Genetic differentiation (F_{ST}) of *Nematalosa erebi* based on 696 SNPs in sites within the Barwon-Darling River (BD), the Warrego River (WR) and the Murray River (MR). F_{ST} values are in the lower triangle, *p*-values from permutation tests are in the upper triangle. Pairwise comparisons showing significant differentiation ($p < .05$) are in grey.

	BD1	BD2	BD3	BD4	BD5	BD6	BD7	BD8	MR	WR
BD1		0.000	0.490	0.155	0.214	0.571	0.000	0.351	0.000	0.126
BD2	0.016		0.651	0.000	0.000	0.272	0.000	0.000	0.000	0.000
BD3	0.000	0.009		0.000	0.000	0.576	0.905	0.000	0.001	0.000
BD4	-0.001	-0.001	-0.004		0.008	0.545	0.132	0.685	0.000	0.003
BD5	0.003	0.002	0.004	-0.001		0.896	0.000	0.115	0.958	0.000
BD6	0.031	-0.001	0.013	0.004	0.013		0.000	0.002	0.000	0.000
BD7	0.039	0.000	0.020	0.010	0.020	-0.004		0.982	0.001	0.000
BD8	0.003	-0.004	0.001	-0.006	-0.006	0.004	0.011		0.795	0.000
MR	0.105	0.079	0.058	0.062	0.079	0.045	0.064	0.068		0.000
WR	0.012	0.012	0.028	0.020	0.010	0.036	0.035	0.022	0.133	

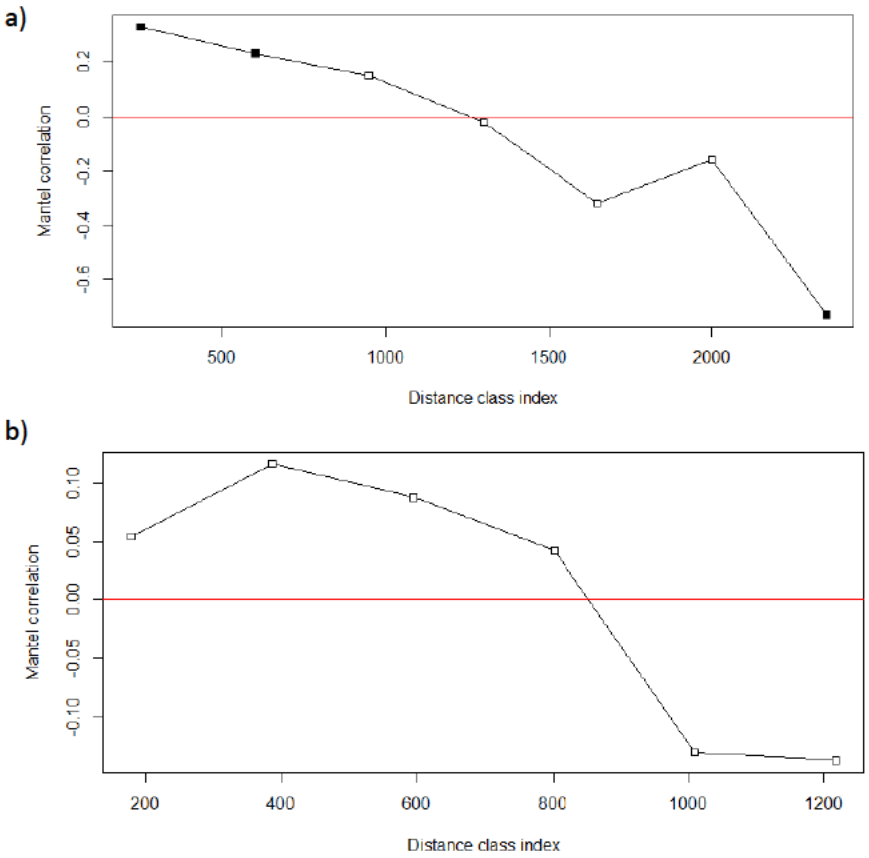

Figure 6-3 Mantel Correlograms *Nematalosa erebi* based on 696 SNPs. a) Results from sites within the Barwon-Darling River, the Warrego River, and the Murray River; b) results from sites within the Barwon-Darling River only. Distance class based on waterway distance (km). Significance was assessed with Pearson's correlation and 10,000 permutations. Filled squares indicate significance ($p < .05$) after Holm correction.

6.5 Discussion

To our knowledge, this is the first study to identify a SNP dataset utilizing DArTseq technology for *N. erebi*. The information obtained from the SNP dataset revealed a well-connected population with no evidence of adaptive divergence. There was no to low differentiation among sample sites, and no apparent influence of landscape resistance on genetic connectivity. Indications of population differentiation were primarily driven by remote populations from the Warrego and Murray Rivers. Sample sizes used in this study were small due to low numbers of fish caught at some sites. The filtered SNP dataset used for analysis was also relatively small after removing loci with MAF <0.05. The sample sizes were likely not large enough to fully characterize local allele frequencies is a limitation of this study.

Isolation-by-Distance & Resistance

Life-history traits suggest that *N. erebi* has the potential to exhibit a panmictic population structure (Hughes et al., 2013). However, as evidenced by significant differentiation, this is not the case at this spatial scale. High levels of admixture in DAPC population assignments indicates that there are no distinct population clusters, suggesting some level of gene flow among sites, but not enough to maintain panmixia.

A pattern of Isolation-by-distance (IBD) occurs when there is a linear relationship between genetic differentiation and geographic distance (Kimura & Weiss, 1964; Wright, 1943). Nearby populations are more connected, with connectivity decreasing along with geographic distance. A pattern of IBD is only evident when the Murray River group is included. A phylogeographic study across the distribution range of *N. erebi* indicated that there is genetic distance between populations in the Murray River and populations in the upper Darling River, but populations in the Murray-Darling Basin are closely related to each other and to neighbouring catchments (Bostock, 2014). Within the Barwon-Darling River there is no linear relationship between geographic and genetic distance. The lack of correlation between genetic and geographic distance suggests that there is no true pattern of IBD and that populations may not be at equilibrium between gene flow and drift (Hutchison & Templeton, 1999). Within the Barwon-Darling River, geographic distance alone does not explain the underlying genetic structure.

Genetic structure was not explained by landscape resistance to movement between sites at this spatial scale. This is potentially due to the relatively short amount of time since the landscape has been altered. There are 15 major weirs on the Barwon-Darling main channel. The oldest weir at Bourke was opened in 1897, the others were constructed between 1960 and 1987 (NSW DPI, 2019). There has also been a rapid increase in water extraction for irrigation since 1960, which has exacerbated the effects of fragmentation due to weirs (Thoms & Sheldon, 2000). Temporal lags in patterns of genetic structure are expected after a change in landscape structure (Landguth et al., 2010), and even low rates of migration are sufficient to maintain signals of genetic connectivity (Lowe & Allendorf, 2010). SNP markers are capable of detecting genetic effects of relatively recent landscape change (Brauer & Beheregaray, 2020) and fine-scale genetic structure in populations expected to be unstructured (Schmidt, A Huey, & Hughes, 2018). However, if genetic connectivity has been reduced due to the presence of weirs, there have not been enough generations to see the impact reflected in the genetic markers. Results of this study do not indicate that genetic structure of *N. erebi* has been shaped by recent landscape modification.

Other widespread Australian fish species with strong dispersal ability show similarly low genetic differentiation. Spangled perch (*Leiopotherapon unicolor*), Australia's most widespread freshwater fish, exhibits low geographical genetic divergence across its range (Bostock, Adams, Laurenson, & Austin, 2006). Landscape genomic studies using SNP markers have found that widespread species have low genetic structure, but they do show evidence of adaptive divergence within the Murray-Darling Basin. Murray Cod (*Maccullochella peelii*) has a panmictic population structure where connectivity is high, with terminal wetlands acting as weak barriers to genetic connectivity, but within the population there is some climate related polygenic adaptation (Harrisson et al., 2017; Rourke, McPartlan, Ingram, & Taylor, 2011). Additionally, Attard et al. (2018) found low to no population structure for golden perch (*Macquaria ambigua*), but did find evidence of adaptive divergence associated with environmental heterogeneity and ecological disturbance. Outlier loci, an indication of loci putatively under selection, were not found in *N. erebi* at this spatial scale. In these widespread species with good dispersal capability ecological attributes have a stronger influence on genetic structure than relatively recent fragmentation.

Species traits influence responses to habitat fragmentation and species with low mobility or that are habitat specialists are expected to be more susceptible to the effects of fragmentation (Ewers & Didham, 2006). Landscape genetics studies of fish species with poor dispersal abilities have revealed fine-scale genetic structure of populations. Genetics structure of the Southern pygmy perch (*Nannoperca australis*) was influenced by both hydroclimatic-driven selection and recent anthropogenic fragmentation (Brauer, Hammer, & Beheregaray, 2016; Brauer & Beheregaray, 2020). Similarly, populations of the river blackfish (*Gadopsis marmoratus*) are isolated with no contemporary gene flow between them as a result of both life history and recent anthropogenic habitat fragmentation (Coleman et al., 2018; Lean et al., 2017).

Metapopulation theory

The pattern of genetic differentiation (F_{ST}) that does not correlate to geographic or resistance distance between sites, the absence of IBD among main channel populations, and the dynamic environment suggest that this species is likely structured as a metapopulation (Huey, Schmidt, Balcombe, Marshall, & Hughes, 2011; Koizumi, 2011). A metapopulation is a set of local populations that are connected through gene flow or dispersal (Hanski & Gilpin, 1991; Levins, 1969). In metapopulation theory, subpopulations experience recurring extinction-colonization events. Dispersal from occupied habitat patches can rescue a population from extinction, or recolonize a habitat patch after extinction (Hanski, 1998). Recurring extinction-colonization events and high gene flow may act to prevent strong genetic differentiation among subpopulations, or increase

differentiation due to drift (Harrison & Hastings, 1996). Species persistence through metapopulation connectivity is a common strategy in extinction-prone environments (Falke & Fausch, 2010; Hanski et al., 2017) such as temporary rivers (Larned et al., 2010). Metapopulation dynamics have been identified in several freshwater biota in the highly variable environment of the Murray-Darling Basin (e.g. *Nannoperca australis*: Cole et al. (2016); *M. ambigua*: Beheregaray et al. (2017); Huey et al. (2011); M. australiense: Huey et al. (2011); *Duma florulenta*: Murray, Reid, Capon, and Wu (2019)). Following metapopulation strategies has likely allowed these species to persist through many environmental changes.

Australia has a relatively depauperate fish community, due in part to its continental isolation (Pollard, Ingram, Harris, & Reynolds, 1990). Within the Murray-Darling Basin only 46 native fish species have been described (Lintermans, 2007). Adaptations of extant biota in Australia reflect the long-term filtering effect of extreme climate shifts and increasing aridity (Butcher, McDonald, & Bell, 2009; Byrne et al., 2008; Morton et al., 2011). Common, widespread species within the Murray-Darling Basin possess traits which convey resistance and resilience to drought (Baumgartner, Wooden, Conallin, Robinson, & Thiem, 2017; McNeil, Gehrig, & Sharpe, 2013). For example, *N. erebi* reproduce during periods of low to no flow (Kerezsy, Balcombe, Arthington, & Bunn, 2011), and are highly fecund (Balcombe et al., 2006; Growns, 2004), allowing them to maintain high populations during drought. They are also capable of large-scale movements (Marshall et al., 2016) and are early colonisers to newly reconnected habitats (Puckridge et al., 2000). These adaptations allow species to survive and recover from seasonal and supra-seasonal drought. However, traits that make species resilient to climate extremes and hydrological variability also may mean that responses to fragmentation are delayed and hard to detect.

In dynamic environments, species may exhibit lagged responses to chronic fragmentation and habitat loss (With, 2004). Particularly in metapopulations where species' responses to habitat loss may be delayed when colonization potential exists only slightly below the threshold for persistence (Hanski, 1998). A species in a metapopulation can tolerate habitat fragmentation so long as the rate of habitat fragmentation does not exceed the scale of that species' metapopulation dynamics (DeWoody, Feng, & Swihart, 2005). We cannot conclude that functional connectivity of *N. erebi* has been reduced, but the lack of a genetic response does not mean lack of impact. Maintaining population connectivity is important for resistance and resilience as the maintenance of standing genetic variation and the spread of beneficial alleles promotes adaptation to novel environments (Barrett & Schluter, 2008). Additionally, species are only able to persist when their habitat persists.

Threats to population

Refugia are important for species to resist the effects of drought and to ensure their capacity to recover after drought (Lake, 2003). Projections of increased aridity in combination with consumptive use of water resources threatens the persistence of aquatic refugia (Larkin, Ralph, Tooth, Fryirs, & Carthey, 2020; Sheldon et al., 2010). Extended periods of disconnection alter biological assemblages in isolated waterholes, shifting from displaying high species diversity towards only supporting a community of tolerant generalists (Sheldon, 2005), with the increased isolation preventing colonizing events (Arthington, Olden, Balcombe, & Thoms, 2010). Water quality in waterholes also reflects localised conditions, showing a decrease in water quality with increased isolation (Sheldon & Fellows, 2010). Metapopulations should be less vulnerable in large waterholes because the probability of drying is lower under natural conditions (Datry et al., 2017). However, low-level weirs have altered the depth and distances between waterholes in the Barwon-Darling River (Pearson et al., 2020). Outside of weir pools, the depth of waterholes has decreased and the distance between deep waterholes has increased (Pearson et al., 2020). Regardless of adaptations to survive drought conditions, organisms cannot survive if habitat is lost.

6.6 Conclusion

N. erebi is an ecologically important but understudied species in the Barwon-Darling River. There is small but significant genetic differentiation between sites within the river that is not explained by distance or resistance to movement. We cannot conclude that reduced structural connectivity due to weirs and altered flow has reduced functional connectivity within this species. However, the change in hydrological connectivity has only occurred in the relatively recent past and any impacts may not yet be reflected in genetic markers. Additionally, strategies enabling species persistence in the variable dryland river environment may mask early indicators of the effect of fragmentation on the population. Landscape genetics provides one approach to assessing the impact of fragmentation on a population, but in a species with strong dispersal ability additional tools may be necessary to assess impacts. Understanding current patterns of genetic structure will allow us to better predict population responses to future environmental change.

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Synthesis and general discussion

7.1 Summary of major findings

The aim of this thesis was to investigate how anthropogenic fragmentation in the Barwon-Darling River has influenced fish. Two indicators that reflect different spatial and temporal scales were used to identify a response to fragmentation: 1) food webs and 2) genetic structure. Food webs measure trophic interactions within the biological community and are expected to be influenced by fragmentation at multiple scales, reflecting recent impacts. Genetic structure of a population is influenced by longer-term processes and incorporates any effects of fragmentation over a longer timescale. Spatial variation of food web structure among sites within the river was not consistent with effects of water resource development and genetic structure within the *N. erebi* population did not indicate reduced functional connectivity. Overall, no correlations were found that can be attributed to a change in the level of fragmentation. The dynamic nature of dryland rivers complicates efforts to understand the response of the biological community to anthropogenic fragmentation. Species that have persisted in these variable environments are adapted to extended periods of natural fragmentation, making any response to anthropogenically exacerbated fragmentation difficult to detect. This study has helped illustrate that assessing the functional response of fishes to water resource development in dryland rivers is complicated, and if there has been a response, different indicator metrics are necessary to detect it.

Chapter three identified important gaps in our knowledge about the fish community response to physical fragmentation in rivers. The bias in the research has been towards rivers in temperate climates and short-term studies of structural response variables. Attempts to use the literature to test specific hypotheses about the response to fragmentation highlighted the need for more holistic studies which identify and quantify the change in connectivity to help us understand the mechanistic links between anthropogenic fragmentation and the biotic response. It also supported the choice of food webs and genetic structure as indicators of anthropogenic fragmentation to fill a knowledge gap.

Chapter four established that non-lethally collected fish fin tissue can provide the same information as lethally collected muscle tissue for stable isotope studies of food webs. This enabled the use of fin tissue in the food web study of chapter five, and will be of value to future studies in the Barwon-Darling River and elsewhere.

Chapter five investigated if spatial variability among fish-centred food webs within main channel habitats of the Barwon-Darling River was related to effects of water resource development. No

patterns were found between spatial variation of food web structure and varying hydrological character of the study sites. A more thorough investigation of food web structure including identification of basal resources and environmental conditions is needed to understand the causes of spatial variation among sites.

Chapter six investigated the genetic structure of *N. erebi*, a species providing an ecologically important role in nutrient transfer, but which has been understudied in the Barwon-Darling River. There was low but significant differentiation among some study sites, but there was no positive correlation with geographic distance between sites. Current population structure indicates that this species may persist as a metapopulation, where persistence relies on connectivity among local populations. Water resource development and increased fragmentation of the river has occurred in relatively recent history in terms of detecting a genetic response, which may be difficult to detect in a widespread metapopulation regardless of time. While no conclusions could be made about a change in functional connectivity within the river, this study does improve our understanding of the underlying population structure of this species.

7.2 Species' adaptations to dryland river environments

Extant biota within the Barwon-Darling River have survived through periods of increasing aridity of the continent (Byrne et al., 2008; Unmack, 2001). Long-term filtering of biological traits and the inherent spatial and temporal variability of dryland rivers has resulted in a species pool of fishes that is resilient and resistant to disturbance such as drought (McNeil, Gehrig, & Sharpe, 2013; Sheldon, 2005). Traits such as flexible life history and feeding strategies, as well as high dispersal capacity, have allowed species to persist in variable and unpredictable dryland river environments. For example, a flexible life-history strategy allows many fish species to recruit independent of flow conditions (Kerezsy, Balcombe, Arthington, & Bunn, 2011). The 'low flow recruitment hypothesis' explains that some species recruit during the more predictable low flow periods in the Murray-Darling Basin (Humphries, King, & Koehn, 1999), allowing them to maintain populations within refugial waterholes and quickly re-populate new habitats (Balcombe et al., 2006; Balcombe & Arthington, 2009).

Some of the traits that make species resistant and resilient to disturbance also make any response to anthropogenic fragmentation difficult to detect and differentiate from baseline conditions. Opportunistic and generalist feeding strategies are widespread among species and enable dryland river fish to exploit resources when new habitats become available and conserve resources while

isolated in waterholes for an unpredictable duration (Jardine et al., 2012; Pusey, Arthington, Stewart-Koster, Kennard, & Read, 2010). Early responses to anthropogenic fragmentation limiting access to resources may be no different than responses to natural drought. Additionally, strong dispersal capacity and maintaining genetic connectivity within the larger population has likely enabled persistance of these species in the extinction-prone dryland river environment (Hanski et al., 2017). Widespread species in the Murray-Darling Basin show moderate levels of phylogeographic structure (Unmack, 2013) and patterns of genetic structure that suggest strong connectivity across large spatial scales (Attard et al., 2018; Bostock, Adams, Laurenson, & Austin, 2006; Harrisson et al., 2017). The ability of these species to take advantage high-flow periods when they do occur may limit any early indication of a genetic response.

Drought, including infrequent periods of extreme drought, is a component of the regional climatic history (Freund et al., 2017; Godfree et al., 2019) to which species have adapted. However, water resource development exacerbates effects of natural drought and impairs the ability of biotic communities to recover when flows resume (Bond, Lake, & Arthington, 2008). Species inhabiting intermittent rivers have adapted strategies to survive throughout variable and unpredictable 'boom' and 'bust' cycle (Bunn, Thoms, Hamilton, & Capon, 2006). However, their persistence during 'bust' periods relies on the maintenance of suitable environmental conditions within refugia and the productive 'boom' periods to recover and prepare for the next period of scarcity (Baumgartner, Wooden, Conallin, Robinson, & Thiem, 2017). Water control structures and extraction of water for consumptive use creates 'artificial droughts' (Boulton, 2003) which extend or result in periods of unnaturally low or no flow. In the short-term, however, biotic responses to 'artificial droughts' would be similar to natural droughts, making responses to anthropogenic-induced fragmentation difficult to detect.

These extended and repeated periods of low flow affect the structure of macroinvertebrate and fish assemblages (Arthington, Balcombe, Wilson, Thoms, & Marshall, 2005; Finn, Boulton, & Chessman, 2009; Leigh, 2013). For example, periods of extended drought can reduce fish recruitment during subsequent flood events (Balcombe, Arthington, Thoms, & Wilson, 2011; Cruz et al., 2020). Returning to drought conditions before the population structure has had time to recover will then influence assemblages within refugial waterholes over the next low-flow period. Legacies of previous conditions influence recovery of a population when flows return (Thompson, King, Kingsford, Mac Nally, & Poff, 2017), and repeated series of droughts without population recovery time reduces population sizes and increases extinction risk (Bond et al., 2015). The overall reduction of flows and flow variability due to water resources development in the Barwon-Darling River (Mallen-Cooper & Zampatti, 2020) may impair the ability of the biotic community to adequately recover to a 'healthy'

baseline status. The additive effects of prolonged or frequent low-flows may push populations beyond the point of recovery before we've detected a response different to what would be expected in a natural drought (Figueiredo, Krauss, Steffan-Dewenter, & Sarmento Cabral, 2019). Adaptations to times of scarcity have allowed these fish to persist, but those adaptations hinder our ability to assess the longer-term effects of anthropogenic fragmentation.

The assessment of food web structure in this study was limited to four species, including one non-native, that are tolerant generalists and have persisted even in harsh habitat conditions at some of the study sites. Species with these traits are unlikely to be sensitive indicators to the effects of increased fragmentation, although the fact that these four species were the only species consistently caught (in addition to one other non-native species) may be an indication itself. The lack of an adequate baseline for assessing food web structure also makes detecting a change challenging. Food web structure reflects community interactions and antecedent hydrology (Robson et al., 2017; Reid, Delong, & Thoms, 2012 but detecting a food web response to increased fragmentation will require identifying more sensitive food web indicators and gaining a better understanding of baseline structure. The cumulative influence of smaller-scale events affects longer-term processes such as maintaining genetic diversity, which relies on species survival and recruitment to disperse and recolonise habitats. A widespread species capable of large-scale movement, such as *N. erebi* is not a sensitive indicator to detect an early genetic response to fragmentation. However, knowing that the capacity for large movements has likely allowed this species to persist means that responses to the smaller-scale processes that promote dispersal and recruitment are better indicators than detecting a genetic response.

7.3 Conclusion

Resistance of biota to drought conditions and their ability to recover when conditions improve is beneficial in terms of their ability to persist but makes it difficult to detect if there is an early response to anthropogenic fragmentation. The potential for a delayed response of this highly adapted fish assemblage means we risk interpreting a lack of detectable response as meaning there are no negative consequences to increasing fragmentation (Sheldon, Thoms, Berry, & Puckridge, 2000). The biocomplexity of rivers requires accounting for legacies of past condition, lagged responses, and species' traits in order to understand the responses, or lack of a response, of the biological community (Thompson et al., 2017).

Incorporating the natural variability of dryland rivers into their management is essential to maintain ecosystem function (Acreman et al., 2014; Arthington, Kennen, Stein, & Webb, 2018). However, incorporating this complexity into monitoring and adaptive management strategies is challenging (Horne et al., 2019; Thompson, Bond, Poff, & Byron, 2018). The results of this study confirm that differentiating between a normal response to drought and a variable environmental, and an early response to the effects of anthropogenic fragmentation is difficult. The ability to detect if the biological community is responding to anthropogenic fragmentation requires an understanding of baseline variability and the use of appropriate indicators. The choice of appropriate indicator is challenging, may vary by region or environmental conditions, and is determined by the temporal scale of interest (Bunn et al., 2014). Food web structure as an indicator may require the inclusion of additional variables, and genetic structure requires a more suitable species to detect impacts of fragmentation.

Habitat variables and conditions contributing to the spatial differences in food web structure were not identified using the broad classifications of this study design. However, the presence of spatial variation in consumer trophic structure after an extended dry period indicates that ecosystem processes differ among waterholes in the main channel. Incorporating trophic dynamics into environmental monitoring provides a holistic view of community responses to management decisions (Rolls et al., 2017). The results of this research suggest that a more thorough study of the factors shaping food web structure during drought and the effect of flows is warranted to determine if food webs can be used as a tool to monitor responses to environmental flows.

The genetic structure of *N. erebi* did not reflect effects of anthropogenic fragmentation due to water resource development. However, it did confirm that this widely distributed, and ecologically important species exists as a well-connected population. Maintaining connectivity and adaptive capacity within the population is important in the face of unprecedented environmental change.

The Barwon-Darling River is an inherently dynamic ecosystem. Periods of extended drought and hydrological disconnection linked to climate are a component of its flow regime. However, the geomorphology and hydrology of the river have been modified since European settlement and the health of the biological community has declined over this time. Forecasts of water scarcity and a continued demand for water to meet human needs requires that we improve our understanding of how human actions have impacted the ecosystem to allow better management into the future.

7.4 Future research directions

A more thorough investigation of food web structure within the main channel is needed to understand the spatial variation that was found. This study was restricted to four species of fish due to the small numbers of fish caught, as well as other study constraints. A study including primary producers and macroinvertebrates would allow for an assessment of basal resources and better resolution of trophic interactions and potentially identify sensitive indicator taxa. Incorporating these shorter-lived organisms and gut content analysis may be necessary to assess smaller-scale and earlier impacts of fragmentation. Identifying if carbon subsidies from the floodplain are an important component of fish diet or identifying which habitat features promote the primary productivity supporting the food web are important when river flows are being managed. Although the species inhabiting these environments likely take advantage of all food resources available, identifying the important resources to manage for may be easier to monitor than conducting frequent food web analysis.

This study occurred after a prolonged period of no-flow with no baseline reference of food web structure during periods when habitats are connected. Although sites had different long-term antecedent flow conditions and habitat characteristics, isotope values at all sites likely reflected drought conditions. Interactions among species and the flow of nutrients and organic matter, among other things, when these waterholes are connected will ultimately influence their trajectory when they are disconnected. If the future of the river is more frequent no-flow periods, understanding how these connectivity events effect trophic interactions is important. Assessing temporal variability of food web structure and monitoring recovery from drought will help us understand food web dynamics within the river.

Enough time may not have passed to detect a genetic response to fragmentation in this widespread species. Nevertheless, a better understanding of *N. erebi* population structure across the Murray-Darling Basin and neighbouring basins may help clarify underlying genetic structure. Confirming metapopulation dynamics and understanding how this species persists will add to our knowledge of the ecology of this, and other widespread species within the basin. We have a good understanding of genetic structure of other widespread species, and a less mobile and shorter-lived species would be a more sensitive early indicator of a genetic response to anthropogenic fragmentation. While a species with traits that would make it a good indicator species may, based on this study, be difficult to sample in adequate numbers in the main channel, genetic studies of such a species may offer a better chance to detect early declines in functional connectivity.

7.5 References

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