MONASH University

Evolutionary Histories and Futures of the Fishes of the Lake Eyre Basin

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Abstract

Highly-fragmented environments represent a significant challenge for population persistence, and many are expected to become more fragmented into the future. The aim of this thesis was to investigate the role of population connectivity in contemporary and future persistence of populations in highly-fragmented environments, specifically desert freshwaters. Aquatic taxa in desert freshwaters are expected to rely heavily on population connectivity for persistence. A meta-analysis of 133 population genetic studies of aquatic desert fauna revealed that population connectivity declined with increasing scale, but did not differ among arid regions nor taxonomic classes. Population connectivity was found to be most influenced by species' ecology and structural/hydrological connectivity, but the role of these factors in population persistence in such environments is poorly understood.

To explore the drivers of population connectivity, a comparative framework was applied to five diverse fish taxa from a desert freshwater ecosystem: the Lake Eyre Basin (LEB) in arid central Australia. These fish possess different suites of traits defining persistence strategies: resistance (high tolerance of environmental extremes, but low mobility) and resilience (the converse). These strategies are expected to influence population connectivity and levels of genetic diversity, thus contemporary and future persistence. To test hypotheses about persistence, population genomics was used to investigate a large genetic dataset, generated by targeted sequence capture, of >700 anonymous nuclear loci (mean length >500 bp) for each fish taxon.

Population connectivity of the five fishes was characterised across the LEB to elucidate patterns driven by species' ecology and structural connectivity. Within and among rivers, resilient taxa exhibited high population connectivity, while resistant taxa exhibited both high and low population connectivity. For all taxa, connectivity was lower at the greater scale. Among-rivers, population connectivity was more strongly influenced by structural connectivity, including hydrology and environmental variables. Overall, resilient and resistant strategists were both effective at maintaining population connectivity in this extreme and fragmented landscape.

Genetic diversity and evolutionary history of fish populations isolated in the Finke River were determined in order to investigate the influence of a loss of structural connectivity on species with different persistence strategies. Since divergence, migration rates into and out of the Finke were positively correlated with genetic diversity levels. The data suggest that both persistence strategies will be able to retain genetic diversity when some gene flow is present. However, in its absence, some resistant taxa may not be able to maintain evolutionary potential required for future persistence.

Overall, this work demonstrates the importance of species' ecology and structural connectivity to contemporary and future persistence of fishes in the LEB. Resistance and resilience strategies facilitate population persistence, although resistant taxa may be more vulnerable to future climate change and other anthropogenic impacts through loss of population connectivity and genetic diversity. This research illustrates the value of a comparative framework and population genomics to understanding the causes and consequences of population connectivity for persistence, and provides insights that can inform management in highly-fragmented environments.

Publications During Enrolment

Murphy, A. L., Pavlova, A., Thompson, R., Davis, J. and Sunnucks, P. (2015). Swimming through sand: connectivity of aquatic fauna in deserts. *Ecology and Evolution*, 5: 5252–5264. doi:10.1002/ece3.1741

Thesis Including Published Works Declaration

I hereby declare that this thesis contains no material which has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

This thesis includes one original paper published in a peer reviewed journal and one currently under revision for publication. The core theme of the thesis is the evolutionary histories and futures of the fishes of the Lake Eyre Basin. The ideas, development and writing up of all the papers in the thesis were the principal responsibility of myself, the student, working within the School of Biological Sciences under the supervision of Professor Paul Sunnucks and Professor Jenny Davis.

The inclusion of co-authors reflects the fact that the work came from active collaboration between researchers and acknowledges input into team-based research.

In the case of chapters two and six, my contribution to the work involved the following:

Thesis Chapter	Publication Title	Status	Nature and % of student contribution	Co-author name(s) Nature and % of Co-author's contribution*	Co-author(s), Monash student Y/N*
2	Swimming through sand: connectivity of aquatic fauna in deserts	Published	Concept, collection and collation of data, data analyses, and writing first draft, 70%	1) Alexandra Pavlova, concept, data collation and input into manuscript, 15% 2) Ross Thompson, input into manuscript, 5% 3) Jenny Davis, input into manuscript, 5% 4) Paul Sunnucks, input into manuscript, 5%	No No No No
6	Divergence in the desert: the impact of isolation and ecology on genetic diversity and divergence of fishes in an ancient river	Under Revision	Concept, approach and study design, sample collection and laboratory preparation, bioinformatics and data analyses, and writing first draft, 70%	1) Bertrand Gauffre, bioinformatics and data analyses, input into manuscript, 5% 2) Mark Adams, provision of samples, input into manuscript, 1% 3) Angus Duguid, provision of samples, input into manuscript, 1% 4) Michael Hammer, provision of samples, input into manuscript, 1% 5) Alan R. Lemmon, DNA sequencing, input into manuscript, 1%	No No No No No No

				6) Emily Moriarty Lemmon, DNA sequencing, input into manuscript, 1% 7) Dale G. McNeil, provision of samples, input into manuscript, 1% 8) Krystina D. Mossop, provision of samples, input into manuscript, 1% 9) Alexandra Pavlova, data analyses and input into manuscript, 5% 10) Emma Razeng, provision of samples, data analyses and input into manuscript, 1% 11) Ross Thompson, concept and input into manuscript, 1% 12) Peter J. Unmack, provision of samples, input into manuscript, 1% 13) Bob B.W. Wong, provision of samples, input into manuscript, 1% 14) Jenny Davis, concept and input into manuscript, 5% 15) Paul Sunnucks, concept and input into manuscript, 5%	No No No Yes No No No No No

I have renumbered sections of the published paper in order to generate a consistent presentation within the thesis, and removed duplicated sections of the methods of the paper under revision for clarity.

Student Signature: [redacted] **Date:** 14/12/2017

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the student's and co-authors' contributions to this work. In instances where I am not the responsible author I have consulted with the responsible author to agree on the respective contributions of the authors.

Main Supervisor Signature: [redacted] **Date:** 14/12/2017

This thesis is dedicated to the memory of
my father, John Lance Murphy
(9th May 1955 – 6th Oct 2017)

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Chapter One

General Introduction

Population Persistence

Understanding the processes that facilitate the persistence of biodiversity through space and time is one of the great challenges in biology. A large body of work has explored this challenge, yet fundamental knowledge gaps remain. These include how suites of ecological traits in species influence their persistence, genetic diversity and evolutionary potential (Larned *et al*., 2010; Segelbacher *et al*., 2010). Answering these questions will help to address these knowledge gaps and inform a wider understanding of the interactions between ecology and evolution.

Highly-fragmented environments represent a significant challenge for population persistence, particularly in species with restricted modes of dispersal. Many environments are naturally fragmented, such as regions of high elevation in an otherwise flat landscape, and the species that inhabit these regions have evolved traits that facilitate persistence through time and space (Keitt *et al.* 1997; Fagan 2002). However, many of these environments are likely to experience further fragmentation through climate change and other anthropogenic impacts, such as habitat loss and changes in land and water use (Woodward *et al.* 2010; Jaeger *et al.* 2014). Understanding how populations persist in fragmented environments can assist biodiversity conservation and management, through identification of vulnerabilities to increased fragmentation.

In fragmented landscapes, populations are typically divided into a network of subpopulations (Hanski 1999). Each subpopulation is prone to extinction from stochastic events, and persistence of the whole population relies on a balance between rates of extinction and recolonisation. Recolonisation requires demographic connectivity, defined by the contribution of dispersers from one population to the growth rate of another (Lowe & Allendorf, 2010). Persistence through recolonisation can be considered 'contemporary persistence', and here will be differentiated from a species' ability to persist into the future in the face of environmental change ('future persistence').

Future persistence of populations relies on their ability to respond to environmental changes (Sgro *et al.* 2011). Range shifts to more suitable conditions are one potential response, but such movements are often restricted or impossible in highly-fragmented environments (Parmesan 2006; Thomas 2010). Alternatively, populations may adapt evolutionarily to environmental change; this is dependent on a population's evolutionary potential, partially determined by genetic diversity (Frankham 2012; Harrisson *et al.* 2014). As such, future persistence requires a population to maintain adequate genetic diversity. In fragmented environments, genetic connectivity, determined by the effects of gene flow on evolutionary processes, drives maintenance of genetic diversity (Lowe & Allendorf 2010). Genetic connectivity is expected to negate some of the effects of genetic drift, a common cause of diversity loss in small populations (Frankham 2005).

Demographic and genetic connectivity together constitute population connectivity (Lowe & Allendorf 2010). Population connectivity is influenced by the intersection of structural connectivity of landscapes and species' ecology, including the key traits dispersal ability and environmental tolerances (Öckinger *et al.* 2010; Hughes *et al.* 2013). Taxa with similar ecologies within a single fragmented landscape are expected to experience similar patterns of population connectivity and therefore population persistence (Faulks *et al.* 2014). Structural connectivity may differ across a landscape, and can result in differences in population connectivity over a species' range (Hughes *et al.* 2009; Baguette *et al.* 2013).

Desert freshwaters represent a highly-fragmented environment for aquatic taxa, with habitat patches typically isolated by an arid terrestrial matrix. Desert freshwaters are discrete and relatively simple ecosystems, and as such are a useful system in which to investigate population persistence in highly-fragmented environments (Fensham *et al.* 2011). They are also a globally threatened biome predicted to experience significant environmental change over the coming decades from climate change and other anthropogenic impacts (Dudgeon *et al.* 2006; Woodward *et al.* 2010). These impacts are expected to increase fragmentation, creating greater challenges for aquatic biodiversity. Thus understanding persistence in this environment will have direct management implications.

The aim of my PhD was to investigate the role of population connectivity in contemporary and future persistence of populations in highly-fragmented environments. This study helps

address major knowledge gaps in our understanding of the persistence of biodiversity, by testing hypotheses about the role of species' ecology and structural connectivity in contemporary and future population persistence. It also informs the conservation and management of populations in fragmented environments. A desert freshwater ecosystem – the Lake Eyre Basin in arid central Australia – was the arena for in-depth investigations of the population biology of five diverse freshwater fish taxa. In addition to testing hypotheses about general processes, this study explored the contemporary and historical genetic patterns and evolutionary processes of the fishes of the LEB, and identified future challenges to their persistence.

Study System: The Fishes of the Lake Eyre Basin

The Lake Eyre Basin (LEB) is located in the arid centre of Australia, and covers one-seventh of the continent. Like other deserts, the region has a climate characterised by high temperatures and low precipitation, although the latter is unusually variable (Van Etten 2009). This aridity has developed over millions of years, as the region has transitioned from rainforest to its contemporary desert landscape (Byrne *et al.* 2008). Today, none of the LEB's rivers have permanently flowing water, so the only aquatic habitats available for riverine species during drought periods are riverine waterholes (Wager & Unmack 2000). On a global scale, the LEB represents one of the last examples of an unregulated, variable dryland river system, and certainly the largest. Accordingly, it provides an ideal system in which to study contemporary and historical evolutionary processes in naturally highly-fragmented environments. Future environmental changes, driven by climate change and other anthropogenic impacts, are likely to increase aridity and fragmentation within the LEB (Watterson *et al.* 2015; James *et al.* 2017). Therefore, characterising contemporary processes that facilitate persistence of biodiversity within the LEB is important for future conservation and management in the region.

Despite the extreme aridity of the LEB, it is home to a surprising diversity of freshwater fishes (Unmack 2001a). These fishes are evolutionarily distinct from fishes of other desert freshwaters globally, due to the unique derivation of most Australian fishes from otherwise marine families (Humphries & Walker 2013). However, they share many of the same challenges faced by other desert fish faunas, and have evolved similar ecological solutions

that facilitate persistence in a highly-fragmented landscape (Kingsford 2006). The fishes of the LEB range from widespread generalists to short-range specialists, and represent a range of different ecologies. These differences provide an opportunity to explore the role of species' ecology on evolutionary processes. Previous studies of LEB fishes' contemporary and historical evolutionary processes have rarely considered multiple species across the entire basin. Here, a suite of five diverse fish taxa, sampled in all major river systems of the LEB, are studied.

Methodological Approach: Comparative Framework and Population Genomics

In this thesis, a comparative framework and a population genomics approach are applied, offering opportunities to explore species' traits that influence persistence. While comparative frameworks are becoming increasingly popular in studies of ecology and evolutionary biology (e.g. Blanchet *et al.* 2010b; Phillipsen *et al.* 2015), population genomics are rarely applied in studies of multiple taxa in the same systems (Jones & Good 2016). By doing so, this study provides the opportunity to evaluate the utility and efficiency of population genomics for population-level studies of ecology and evolution.

Comparative frameworks allow exploration of important biological questions at greater scales, and are effective for identification of general patterns (Andrew *et al.* 2013). Such a framework is particularly useful for studies of population genetic and phylogeographic patterns, and for determining the species' traits that drive these patterns in a single environment (Mims *et al.* 2017). Pauls *et al.* (2014) note that many studies that utilise a comparative approach find species-specific results instead of general patterns, and suggest that this may result from a reliance on one or a few molecular markers. This is problematic, as single-gene phylogenies reflect the history of the gene, and may not mirror the population history (Edwards & Beerli 2000). In addition, some markers (including allozymes and mitochondrial loci) are likely to be under selection, potentially further biasing estimates of their history (Sunnucks 2000). As these markers are not always representative of genome-wide patterns, and typically only small numbers of loci are used, they may not provide the resolution required to investigate detailed evolutionary histories and contemporary patterns (Andrew *et al.* 2013). To avoid these issues, using many loci is recommended.

Recent advances in molecular techniques offer researchers the ability to utilise multi-locus data on a much greater scale than ever before. This allows exploration of fundamental biological questions that were previously unresolvable due to an inability to generate sufficient genetic data. The issues that arise from using few markers can largely be eliminated by next-generation sequencing (NGS), which has revolutionised the study of ecological and evolutionary biology (Ellegren 2014). NGS represents a powerful suite of tools that enable screening and analysis of large numbers of individual samples and genetic markers (McCormack *et al.* 2013; Goodwin *et al.* 2016).

While NGS was originally developed for whole-genome sequencing (WGS), such an approach is often impractical for population genomic studies (i.e. studies that use a population genetic approach with NGS data; Ellegren 2014). This is because most NGS frameworks require either a reference genome, which rarely exists for natural populations, or *de novo* assembly of the genome, which is often prohibitively computationally-intensive, time-consuming and expensive (Jones & Good 2016). Fortunately, there are a number of genome-partitioning techniques that allow only a subset of the genome to be sequenced, while also reducing the effort and cost involved (Davey *et al.* 2011). These techniques include RAD-seq (Miller *et al.* 2007) and RNA-seq (Wang *et al.* 2009), which have become the most common methods used in evolutionary studies, although they can be limiting for some population genomics studies (Jones & Good 2016).

An alternative is high-throughput targeted sequence capture, which is a cost-effective approach for generating many orthologous loci for many individuals (Olson 2007). This approach achieves genome partitioning through parallel enrichment of preselected regions of the genome (Mamanova *et al.* 2010). Targeted capture produces higher quality data than RAD-seq and RNA-seq, with greater reproducibility and accuracy in SNP calling, and less variance in coverage of targets (Jones & Good 2016). Importantly, it also outputs longer assembled contigs, which provide greater information for sequence-based population genomic analyses (Gnirke *et al.* 2009). Despite these benefits, targeted capture is rarely utilised in studies of ecology and evolutionary biology (Jones & Good 2016). This is partly because of low availability of targeted capture sequencing services, but also because of perceived difficulties in the approach. The greatest challenge is the requirement of *a priori* knowledge of target sequences, which are typically lacking for non-model organisms.

However, a range of solutions has been developed, including using low-coverage WGS of a single individual initially to identify suitable targets (Lemmon *et al.* 2012).

Within this study, I utilise a nuclear sequence dataset of over 700 loci for 785 individuals of five taxa, generated through high-throughput targeted sequence capture. This represents the largest genetic dataset ever assembled for an investigation of LEB fishes, and potentially for any desert fish fauna worldwide, and so can test the value of approaches leading to increased power for answering questions in such systems. A novel laboratory and analytical methodology was developed and implemented with collaborators that allowed samples of different taxa to be pooled together for sequencing, through development of species-specific probes that capture DNA sequence markers unique to each taxon. This technique can greatly improve the cost-effectiveness of high-throughput targeted capture sequence data for diverse taxa simultaneously, facilitating comparative approaches. Evaluating the utility and efficiency of high-throughput targeted sequence capture, the new pooling technique, and the powerful genomic dataset generated, for population-level studies of ecology and evolution is a secondary aim of this thesis, which can be used to inform future research approaches.

Thesis Outline

The aim of this thesis is to explore the evolutionary histories and futures of the fishes of the Lake Eyre Basin (LEB), in order to understand how populations persist in highly-fragmented environments. Each chapter contributes to this aim, as outlined below.

Chapter One

In the present introductory chapter, the rationale for the research, and key background information necessary to understand the studies is explained. The context and approach of the study is also introduced, and the structure of the thesis outlined.

Chapter Two

In Chapter Two, a review of population connectivity patterns of aquatic taxa in desert freshwaters globally is undertaken to synthesise existing research, identify knowledge gaps and set research priorities. This chapter begins with a review of connectivity models applicable to desert freshwaters. A meta-analytical approach is then applied to a dataset of 133 studies, and the patterns in methodology, study systems, and conclusions about population connectivity investigated. The results show that population connectivity is strongly influenced by species' dispersal ability, hydrological regimes, and the scale at which the study was conducted. It is concluded that future studies should estimate gene flow and other population genetic parameters, and use this information to assess models of connectivity. These models are an effective way to understand population connectivity, and, as such, are useful for informing biodiversity conservation and species management in desert freshwaters.

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Chapter Three

Chapter Three reviews the study regions – the rivers of the Lake Eyre Basin (LEB), in arid central Australia – to provide context for the following chapters. The historical processes that have shaped the LEB are explored, and contemporary climate and hydrological regimes discussed. The aquatic habitats of the region, particularly riverine waterholes, are described,

with differences among river systems emphasised. The 'boom and bust' ecology of these desert freshwaters is investigated, given the importance of these processes for native aquatic taxa. Knowledge of the ways in which multiple processes interact in the LEB, including climate, hydrology and ecology, is essential for understanding how freshwater biota persist in this extreme environment. It is concluded that the extreme and dynamic LEB features a number of 'natural experiments' that can be utilised to study many different aspects of population persistence in fragmented environments.

Chapter Four

In this chapter, a review of the fishes of the LEB is conducted, in order to provide context for the following chapters. The LEB fish assemblage is discussed in the context of other desert fish faunas, and its unique origins explored. The five diverse taxa that are studied within this thesis are described in detail, with a strong focus on the biological traits (including environmental tolerance and dispersal ability) that facilitate their persistence in this arid landscape. A framework of 'persistence strategies' is outlined, which comprises a continuum from resistance (high tolerance of environmental extremes, but low mobility) to resilience (the converse). The implications of these different strategies for the evolution of fishes has not previously been investigated, and significant knowledge gaps are identified, including how species' ecology has affected, and will affect their future evolution.

Chapter Five

In Chapter Five, the population genetic structure of five LEB fishes is explored, in order to investigate the consequences of these species' different persistence strategies for population connectivity. A large-scale sampling strategy was applied, with fish collected across the LEB, and next-generation sequencing used to generate a massive dataset of >700 nuclear loci sequences per taxon. The population genetics of each taxon are analysed using a variety of approaches, with levels of genetic diversity, genetic structure, and contemporary gene flow determined. The results indicate that persistence strategies influence population connectivity within and among rivers, with greater levels identified in resilience-strategists. It is concluded that persistence strategies are important drivers of evolutionary processes of LEB fishes, and that these characterisations of species' ecology are useful for both scientific understanding and biodiversity management.

Chapter Six

Chapter Six, explores the effects of a loss of structural connectivity by utilising a 'natural experiment' within the LEB – the isolation of the Finke River by desert expansion. This isolation is expected to have had substantial evolutionary genetic consequences for the fish populations within the Finke, which may differ with species' ecology. To investigate this, five fish taxa, representing two persistence strategies (resistance and resilience), are examined, using a large genomic dataset. The results show that divergence timing and effective population size are not related to strategy, while migration rates and genetic variation are. Overall, it is concluded that both persistence strategies should be able to retain genetic diversity when some gene flow is present. Given likely future declines in structural connectivity in desert freshwaters, this study highlights the importance of maintaining population connectivity for population persistence.

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Chapter Seven

The concluding discussion chapter presents an overview of this first body of work to explore the population genomics of a suite of co-distributed and diverse fish taxa across a desert landscape. The utility of a population genomics approach based on a high-throughput targeted capture strategy is evaluated. The implications of this research, both for scientific understanding of ecological and evolutionary processes in such extreme arid environments, and for informing applied management and conservation of the species that inhabit such environments, are discussed. Finally, some remaining knowledge gaps are highlighted, accompanied by suggestions for future research priorities.

Chapter Two

Swimming through Sand:
Connectivity of Aquatic Fauna in Deserts

Abstract

Freshwater ecosystems in arid regions range from highly fragmented to highly connected, and connectivity has been assumed to be a major factor in the persistence of aquatic biota in arid environments. This review sought to synthesize existing research on genetic estimation of population connectivity in desert freshwaters, identify knowledge gaps, and set priorities for future studies of connectivity in these environments. From an extensive literature search, we synthesized the approaches applied, systems studied, and conclusions about connectivity reached in population genetic research concerning desert freshwater connectivity globally. We restrict our scope to obligate aquatic fauna that disperse largely via freshwaters and exclude those with active aerial dispersal abilities. We examined 92 papers, comprising 133 studies, published from 1987 to 2014. Most described studies of fishes and invertebrates in the deserts of Australia and North America. Connectivity declined with increasing scale, but did not differ significantly among arid regions or taxonomic classes. There were significant differences in connectivity patterns between species with different dispersal abilities, and between spring and riverine habitats at local scales. Population connectivity in desert freshwaters is typically most influenced by the ecology of the species concerned and hydrological connectivity. Most studies did not assess predefined models of connectivity, but described gene flow and/or genetic structure. Climate change and anthropogenic impacts worldwide are likely to increase the incidence and impact of habitat fragmentation in already threatened desert freshwaters. To reduce this risk, biodiversity conservation and environmental management must address connectivity, but often the required information does not exist. Researchers can provide this by explicitly considering the effects of hydrology and species' ecology on connectivity, and incorporating these into connectivity models, which are vital for understanding connectivity in desert freshwaters.

Introduction

Arid and semi-arid regions, here referred to as deserts, cover more than 30% of the world's surface area (Peel *et al.* 2007). They dominate the Australian and African continents, and significant portions of Asia, North America and South America (Fig. 1). Deserts are defined by an annual rainfall of no more than 500 mm and an annual evaporation rate equivalent to 95% or more of this total (Meigs 1953). These environments are among the most inhospitable places on Earth, but almost all contain aquatic habitats. Despite these habitats being typically restricted in number and extent, they are important for many desert species. Desert freshwaters include springs, river networks, lakes and pools that may be ground- or surface-water fed. These range across a continuum of temporal permanence, with many classified as temporary (Kingsford 2006).

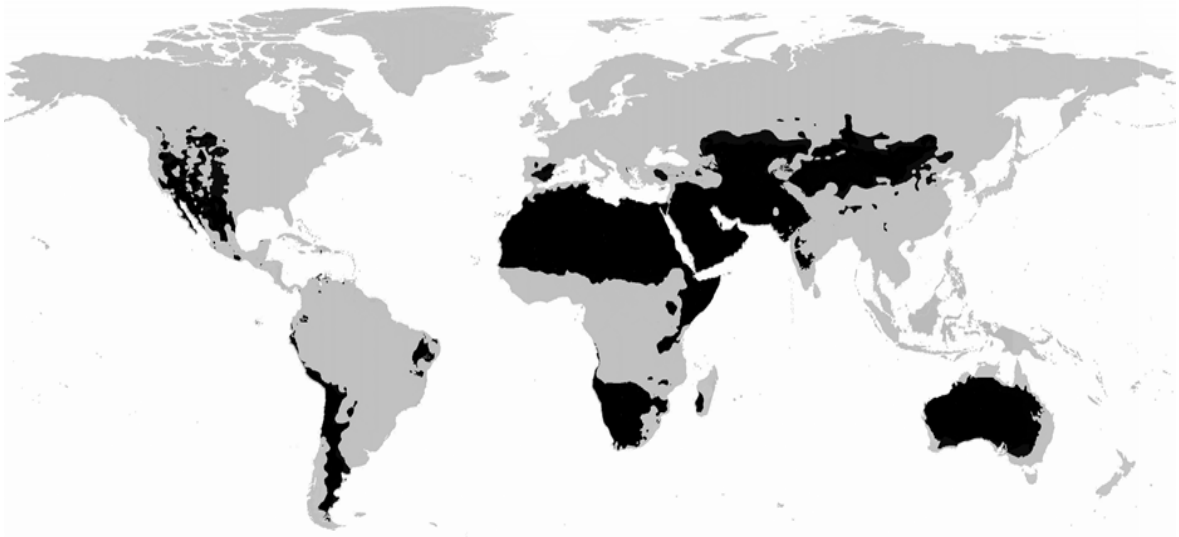

Figure 1. Deserts of the world (black), based on Köppen-Geiger climate data (adapted from Peel *et al.* 2007).

Desert freshwater ecosystems provide vital resources for a wide range of taxa as well as valuable ecosystem services for local people (Kingsford 2006). They provide habitats for aquatic biota, including invertebrates, fishes, amphibians and turtles, and can act as ecological or evolutionary refugia (Davis *et al.* 2013). Despite their importance, these freshwaters and their inhabitants are less well studied than those in mesic environments (Sada *et al.* 2005; Kingsford 2006; Brim Box *et al.* 2008; Vazquez-Dominguez *et al.* 2009). They are also among the world's most threatened biomes, with water extraction, habitat degradation and flow modification directly impacting biodiversity and ecosystem functions (Abell 2002; Dudgeon *et al.* 2006; Palmer *et al.* 2008; Vorosmarty *et al.* 2010). These threats

are intensified further by the natural isolation of freshwater habitats (Bates *et al.* 2008; Davis *et al.* 2013) and global climate change (Woodward *et al.* 2010; Jaeger *et al.* 2014). Together, these threats are expected to further increase the fragmentation of desert freshwaters.

In the face of these threats, effective biodiversity conservation and environmental management is required, and understanding how populations are spatially and temporally connected is imperative for predicting management outcomes (Hermoso *et al.* 2011; Hughes *et al.* 2013). Persistence in fragmented desert freshwaters, which are typically spatially and temporally variable, often requires that species maintain wide geographic ranges to enable dispersal when hydrological connectivity allows (i.e. during floods). Such connectivity is also spatially and temporally variable (Meffe & Vrijenhoek 1988). Unless noted otherwise, we use the term connectivity to refer to population connectivity – the combination of genetic connectivity, determined by the effects of gene flow on evolutionary processes within populations, and demographic connectivity, defined by the contribution of dispersers from one population to the growth rate of another (Lowe & Allendorf 2010).

Species' ecology, physical connections between habitats provided by environmental factors (structural connectivity) and their interactions are the drivers of desert freshwater connectivity (Hughes *et al.* 2013). Relevant species' ecology encompasses many biological factors, including dispersal ability, physiological tolerance, niche breadth and reproductive potential (Öckinger *et al.* 2010). These factors greatly affect how a species is distributed, under what conditions it can persist, and how its distribution can change. Many desert-dwelling freshwater species are highly tolerant of environmental extremes, such as high temperature, salinity, etc. (e.g. Glover 1971; McNeil *et al.* 2011b), and many have excellent dispersal abilities (e.g. Stanley *et al.* 1994; Unmack 2001a; Fagan *et al.* 2002). Such characteristics may also allow them to take advantage of typically limited structural connectivity.

In freshwater ecosystems, geomorphology and hydrology are the most important environmental factors determining structural connectivity. Geomorphologically, rivers and most other aquatic habitats in desert regions do not differ significantly from those in wetter regions (Nanson *et al.* 2002). However, hydrologically, dryland freshwaters are far more variable than those in mesic areas, and often experience long periods without flows, leading

to disconnection (Fig. 2) and reduced structural connectivity (Carini & Hughes 2006). There are also differences in hydrology between deserts. For example, rivers in Australian deserts are almost entirely reliant on a rainfall regime that is among the most variable and unpredictable in the world (Van Etten 2009). In contrast, many North American desert freshwaters are fed by more seasonal inputs, including snowmelt, meaning that temporary flows are somewhat predictable (e.g. Bogan & Lytle 2007).

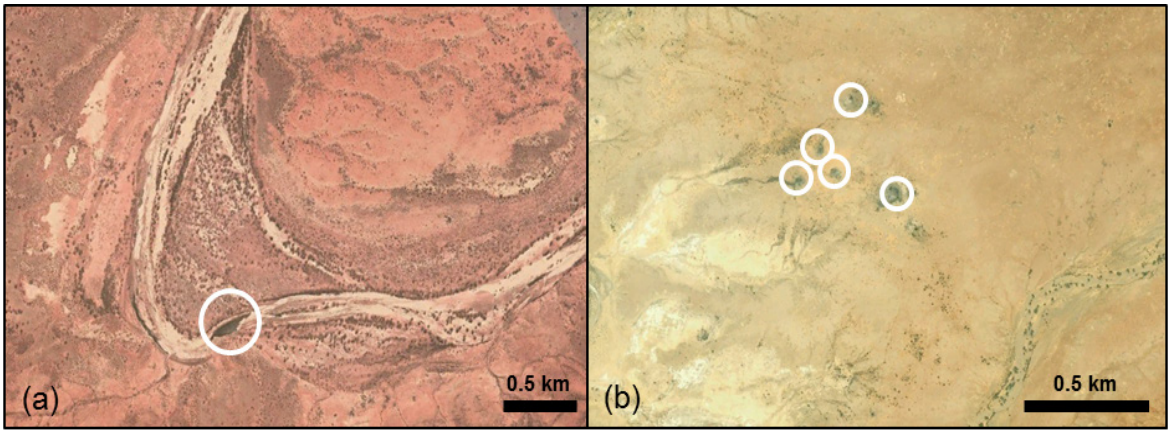

Figure 2. Examples of structurally disconnected freshwater habitats (circled) in arid central Australia: A) a disconnected waterhole on the Finke River (24.58°S 133.30°E), and B) disconnected spring outflows in the Hawker Spring complex (28.41°S 136.18°E). Source: Google Earth.

Here we conduct a review of studies of connectivity of obligate aquatic species in arid regions worldwide, to inform future research and conservation of desert freshwater biodiversity. Our aim is to provide the first synthesis of methodologies and results in this field, by using an increasingly common and appreciated quantitative approach that allows strong conclusions to be made from a diverse literature (e.g. Beheregaray 2008; Storfer *et al.* 2010). We begin by reviewing the connectivity models that can be utilised in desert freshwater connectivity research, and then integrate knowledge of desert freshwater connectivity across regions, scales, habitat types, taxonomic classes and dispersal abilities. We restrict our review to taxa that generally require freshwaters for dispersal, and exclude species with active aerial dispersal mechanisms. We focus exclusively on studies that use molecular data, as genetic estimates of population history offer an increasingly cost-effective approach to understanding past and present connectivity that cannot be practically done any other way for many locations and organisms.

Connectivity Models for Desert Freshwaters

Connectivity models (also referred to as population structure or genetic structure models) are utilised in many connectivity studies, and have been extensively used in freshwater research. Wright's (1943) Panmixia and Isolation by Distance were the first connectivity models. The former proposes that a species is able to disperse easily across its entire distribution, meaning its populations are highly connected and genetically homogenous (see Table 1 for details of the population genetic structure of each model). In contrast, Isolation by Distance (IBD) proposes a decrease in genetic similarity with geographic distance throughout the distribution of a species, reflecting dispersal limitation. IBD is common in nature, and may be more appropriate as a null hypothesis in connectivity studies than is panmixia, depending on the spatial scale of study relative to the scale on which dispersal occurs (e.g. Amos *et al.* 2014).

Table 1. Models of Desert Freshwater Connectivity (adapted from "Models of Genetic Structure", Hughes *et al*. 2013).

Model	Population Genetics Description	Reference
Panmixia	No genetic structure among populations, extensive gene flow.	Wright 1943
Isolation by Distance	Genetic structure between populations strongly correlated with geographic distance.	Wright 1943
Isolation by Resistance	Genetic structure between populations strongly correlated with resistance distance (a measure of gene flow likelihood between two locations).	McRae 2006
Isolation by Environment	Genetic structure between populations correlated with environmental heterogeneity and not geographic distance	Wang & Bradburd 2014
Stream Hierarchy Model	Genetic structure between populations strongly correlated with the physical structure of the stream network.	Meffe & Vrijenhoek 1988
Headwater Model	Genetic structure between populations of headwater specialists strongly correlated with geographic distances in headwaters.	Finn *et al*. 2007
Death Valley Model	Strong genetic structure between populations resulting from loss of connectivity, no contemporary gene flow.	Meffe & Vrijenhoek 1988

Owing to the hierarchical nature of many aquatic habitats and the spatially and temporally disconnected flows in many deserts, specialised connectivity models have been developed for desert freshwaters. Meffe and Vrijenhoek (1988) proposed two models to depict

connectivity in North American desert freshwaters, the Stream Hierarchy and Death Valley models. The Stream Hierarchy Model states that patterns of connectivity should follow the dendritic patterns of the stream network, incorporating both geographic distance and habitat connectivity (Hopken *et al.* 2013; Hughes *et al.* 2013). In contrast, the Death Valley Model assumes extremely low connectivity between sites and high, spatially unstructured differentiation among populations, and thus no relationship between geographic and genetic distance.

A small number of specialised connectivity models extended these early ones, and can be applied to desert freshwaters. These include the Headwater Model, which applies principally to headwater taxa, and assumes presence of temporary aquatic connections between catchment boundaries or some terrestrial dispersal ability. The Headwater Model predicts that species will be able to utilise connectivity between headwaters in adjacent streams, not necessarily in the same catchment, and that these populations will be more genetically similar than those in streams with non-adjacent headwaters (Finn *et al.* 2007; Hughes *et al.* 2009).

Recent approaches have expanded connectivity studies to include variables other than geographic distances. Isolation by Resistance utilises spatially explicit predictive surfaces of connectivity, with landscape resistances to dispersal conditioned on landscape features, which can be compared with predictions from the above models (McRae 2006). A range of landscape variables, in many combinations, can be used to calculate resistance distances (e.g. Cañedo-Argüelles *et al.* 2015; Morán-Ordóñez *et al.* 2015). In contrast, Isolation by Environment offers a framework for examining the effects of ecological and environmental heterogeneity on connectivity, while controlling for the effect of geographical distance (Wang & Summers 2010; Wang & Bradburd 2014; Morán-Ordóñez *et al.* 2015). The expected pattern is one where genetic differentiation increases with environmental differentiation, independent of geographic distance, and is generated by natural or sexual selection against immigrants, reduced hybrid fitness or biased dispersal (Wang & Bradburd 2014). Finally, a number of process-based approaches to test IBE have been built (Wang & Bradburd 2014). These include Isolation by Adaptation (Nosil *et al.* 2008) and Isolation by Ecology (Claremont *et al.* 2011; Shafer & Wolf 2013), but neither have yet been applied to desert freshwaters.

Methods

A dataset of empirical studies was analysed to give an overview of the trends in desert freshwater connectivity research, including methodologies, study systems and results. The dataset was compiled by searching all databases of the Web of Science® collection on 2 March, 2015. The search terms were ('genetic*' OR 'connectivity' OR 'population structure') AND ('freshwater*' OR 'river*' OR 'stream*' OR 'spring*') AND ('desert*' OR 'arid*' OR 'dryland' OR 'rangeland' OR 'temporary' OR 'ephemeral' OR 'intermittent' OR 'fragment*'). To remove results from unrelated fields of study, searches were restricted to five biological categories (Environmental Sciences, Ecology, Evolutionary Biology, Marine & Freshwater Biology, Zoology), and the journal *Conservation Genetics*. Results were refined to include only "articles" (papers).

For inclusion, studies had to analyse population structure or connectivity, using molecular methods, in locations defined as "deserts" as above. Taxa were restricted to obligate aquatic fauna, defined as animals that spend all or most of their life history in freshwater. To restrict our review to taxa that require freshwaters for their dispersal, species with active terrestrial or aerial dispersal mechanisms were excluded, as were those that disperse via marine waters (at least within the studied system). Species that can disperse passively, either aerially or through water, for example via phoresy, wind or in-stream drift, were included. Because of the specialised ecology and connectivity of species living in underground waters (stygofauna), studies of these taxa were excluded.

A total of 3,171 papers were found in the search, of which 70 m*et al*l inclusion criteria. The reference lists of included papers were consulted, and 22 relevant papers were added, resulting in a final dataset of 92 papers (for details and references of all included papers see Appendix 1.1). Several studies examined the same species at the same sites; in these cases only the most recent study was included. To confirm the effectiveness of our search terms, we consulted the database of Australian freshwater connectivity studies compiled by Hughes *et al.* (2013): our search criteria found 13 of the 14 relevant articles included therein.

Publication details were obtained from each paper for analyses of temporal trends. Genetic marker/s and analytical methodologies used to infer or test connectivity or gene flow were recorded to gauge what analyses were possible and the power of inferences (see Table 2 for

definitions of different methodologies). Connectivity models named and/or tested were also recorded (see Table 1).

Where multiple species were included within a paper, each taxon was treated as an additional study, with a final dataset including 133 studies. To check for under-studied topics and compare patterns of connectivity, the following characteristics of each study were recorded: taxonomic class, study region/s, spatial extent (maximum straight-line distance between any two sampling sites), scale, habitat and species' dispersal ability (classifications of the latter three as per Table 2). Red List status was recorded for vertebrates, the only included group to have been largely evaluated by the IUCN (IUCN 2014).

The connectivity models concluded as best fit were recorded for each study to compare the conclusions among different paper approaches and study parameters outlined above. Where different conclusions were reached for different locations or scales within a study, these were recorded as additional conclusions. As many studies considered multiple scales, conclusions were recorded for each scale, giving a total of 141 conclusions. Where papers did not explicitly provide a connectivity model, we categorised their conclusions as no, restricted or high gene flow, based on their descriptions of gene flow or genetic structure.

For statistical analysis, the conclusions made in each study about connectivity (description of gene flow or connectivity model) were grouped into three categories: high connectivity (which included high gene flow and panmixia), restricted connectivity (including restricted gene flow, Isolation by Distance and the Stream Hierarchy model), and no connectivity (no gene flow and the Death Valley model). The prevalence of these categories was compared among the following variables recorded for each study: analytical methodology, scale, region, habitat, taxonomic class and dispersal ability. To test if the conclusions reached differed with these variables, we used a Pearson's Chi-square contingency test in R (R Core Team 2014). Significance of temporal trends of methodologies was tested by calculating Pearson's correlation coefficient in R.

Table 2. Descriptions of study variables recorded and tested for effect on connectivity, and the categories within.

Variable	Description
Analytical Methodology	The methodology used to estimate gene flow.
Deterministic	Deterministic methods included inferences of population structure based on F_{ST} or other genetic distance measures, and nested clade phylogeographic analyses (Templeton 1998).
Probabilistic	Probabilistic model methods included approximate Bayesian computation (ABC; Beaumont 2010), coalescent approaches that estimate levels of gene flow (e.g. IMa (Hey & Nielsen 2004, 2007) and MIGRATE (Beerli 2006); reviewed in Kuhner (2009)), and assignment methods (assignment tests, genetic mixture analyses and parentage analyses; reviewed in Manel *et al.* (2005)).
Habitat	The habitat type in which the study was conducted.
River	Connected surface-fed systems.
Pool	Disconnected surface-fed systems.
Spring	Groundwater-fed systems.
Multiple	A combination of two or more of the above habitat types.
Scale	The hydrological scale at which the study was conducted (note that some studies were conducted at multiple scales)
Within-System	Within a river catchment, or pool or spring complex, local scale, i.e. with freshwater hydrological connections.
Between-Systems	Between river catchments, or pool or spring complexes, within the same basin, i.e. with possible freshwater hydrological connections.
Between-Basins	Between rivers, pool or spring systems, within different basins, i.e. with no freshwater hydrological connections.
Dispersal Ability	The perceived dispersal ability of the species studied, based on descriptions of dispersal in the reviewed papers (not genetic patterns); or, where dispersal ability was not described, based on species' biology or that of related species.
Low	Species with maximum likely dispersal not exceeding the local, within-system scale, e.g. weak-swimming fish, some molluscs.
Moderate	Species with maximum likely dispersal not exceeding the between-system scale, e.g. strong-swimming fish, invertebrates with drifting larval stage.
High	Species with maximum likely dispersal at the between-basin scale, e.g. taxa with passive aerial or terrestrial dispersal abilities.

Results

Publications

Relevant papers included in our review were periodically published from 1987 until the early 2000s; publication rates increased through to 2010, and declined post-2010. The majority of papers were published by lead authors in the USA (47%) and Australia (29%), with the rest from Portugal (10%) and ten other countries, including Brazil, Chile, and eight European countries (15%). Papers were published in 30 different journals, most in discipline-specific journals, the most common being *Molecular Ecology* (21%), *Freshwater Biology* (14%) and *Conservation Genetics* (12%).

Methodologies

A total of eight molecular marker classes were used to assess connectivity. The most common marker was mitochondrial DNA (mtDNA; 66% of papers), followed by microsatellites (36%), allozymes (22%), nuclear DNA sequences (nDNA; 9%), amplified fragment length polymorphisms (AFLP; 8%), and the remaining classes, restricted fragment length polymorphisms (RFLP), randomly amplified polymorphic DNA (RAPD) and single-primer amplification reaction (SPAR), were each used in fewer than 4% of papers. Most papers (55%) utilised one class of marker, 41% used two classes, and 3% used three classes.

There was a strong temporal component to which genetic marker classes were applied. Allozymes were the only markers used until 1996. From 2001, mtDNA and microsatellites became the main markers, with RFLP, AFLP and RAPD used mostly (but rarely) from 2001 to 2006. Studies utilising nDNA sequences first appeared in 2010. The number of marker classes used showed, at most, a small increase over time ($r^2 = 0.04$, $p = 0.05$), while the number of individual loci used showed no significant trend ($r^2 = 0.03$, $p = 0.10$). Almost 19% of papers used just one locus, in most cases mtDNA. At the other end of the scale, 15% of papers used 15 or more loci, generally allozymes.

Analytical methods fell into two main groups – 60% of papers estimated gene flow using deterministic models, including F_{ST}, other genetic distances or genetic structure (nested clade phylogeographic analyses was included in this category), while 40% used probabilistic models, including coalescence, approximate Bayesian computation, and/or assignment

methods. The analytical methodology used showed a strong change over time. The proportion of papers using deterministic models showed a significant decline ($r^2 = 0.52$, $p < 0.001$). In contrast, probabilistic models showed a significant increase in usage ($r^2 = 0.52$, $p < 0.001$) from 2003 and have been used in more studies since 2011.

Most studies did not consider a range of connectivity hypotheses, and Isolation by Distance and Panmixia were almost always the only ones explicitly tested. The Stream Hierarchy and Death Valley models were the next most common models tested (18% and 3% of all studies respectively); while Isolation by Resistance was tested in just two studies (2%). There were no studies testing the Headwater Model, Isolation by Environment, or the other 'Isolation by' models in the included papers, although the Headwater model was tested in one superseded paper (Finn *et al.* 2007). Overall, 25% of papers identified a best-fit connectivity model for their system, while the rest described only the degree of gene flow (51%) or genetic structure (24%). The proportion of papers testing connectivity models showed no significant change over time ($r^2 = 0.07$, $p = 0.23$). Eighteen percent of papers based on deterministic models concluded a connectivity model, compared with 35% of those based on probabilistic models.

Study Systems

Of the 92 papers, 77% examined just one taxon, 12% two taxa, and 11% studied three or more taxa to a maximum of six. Overall, 107 species were included, of which 45% were vertebrates (fish, amphibians and reptiles). Of the vertebrates, 46% were threatened species, 28% non-threatened, and the rest unevaluated (IUCN 2014). The 133 included studies incorporated nine taxonomic classes, although five of these (Bivalvia, Ostracoda, Insecta, Amphibia and Reptilia) together accounted for just 10.5% of studies (Fig. 3A). Species with high dispersal abilities (11% of studies) were less studied than those with moderate (42%) or low (47%) dispersal abilities.

The vast majority of studies were restricted to one of twelve countries (Fig. 3B), with just 4% of studies crossing international borders. Studies were almost always conducted in developed countries, principally the USA and Australia, with smaller numbers in Europe. The studies incorporated eight global arid regions, but 94% of studies were conducted in just three – North American deserts (42% of studies), the Australian arid zone (41%), and

Mediterranean Basin (11%). The spatial extent of studies ranged from 1 to 3500 km (mean 484 km). Most studies were performed within riverine (54%) or spring (34%) habitats, with a small number in pools (5%) and multiple habitats (7%).

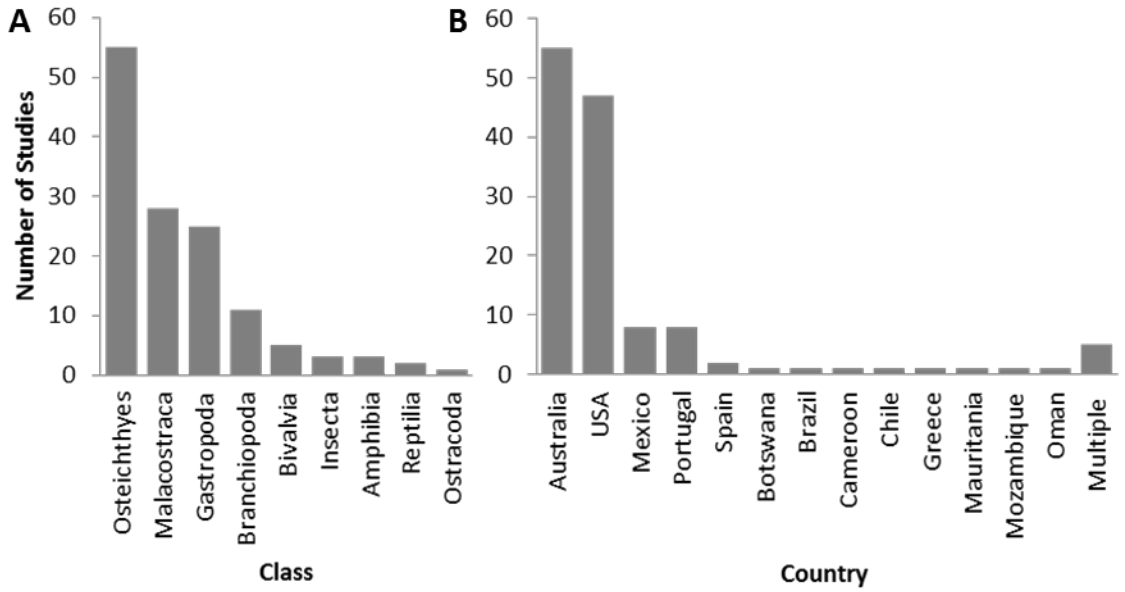

Figure 3. Number of studies examined of connectivity in desert freshwaters according to A) class of study taxa, and B) country of study.

Most studies considered several scales; 19% considered all three (see Table 2 for scale descriptions) within river/spring system, between river/spring systems, and between river/spring basins); 41% considered two (mostly the two smallest scales, 67%); and 40% considered only one (predominantly, 75%, the within-system scale). Overall, 80% of studies examined connectivity at the within-system scale, 61% at the between-systems scale, and 37% at the between-basins scale.

Connectivity Patterns

A clear pattern of decreasing connectivity at larger spatial scales was apparent (Fig. 4). When all conclusions about connectivity were combined into three categories (high, restricted, none), connectivity was found to differ significantly between the three scales ($\chi^2 = 53.63$, d.f. = 4, $p < 0.0001$). The number of systems with no connectivity increased from 12% at the within-system scale to 69% at the between-basin scale, while the number with high and restricted connectivity decreased as spatial scale increased. The overall connectivity category (i.e. high, restricted, none) concluded in each study did not differ significantly

between studies that used deterministic or probabilistic models, when considering all studies ($\chi^2 = 0.53$, d.f. = 2, $p = 0.77$) or when considering each of the three scales individually ($\chi^2 = 0.25 – 0.53$, d.f. = 2, $p = 0.77 – 0.88$). Therefore, studies were not separated on the basis of analytical methodology when analysed further.

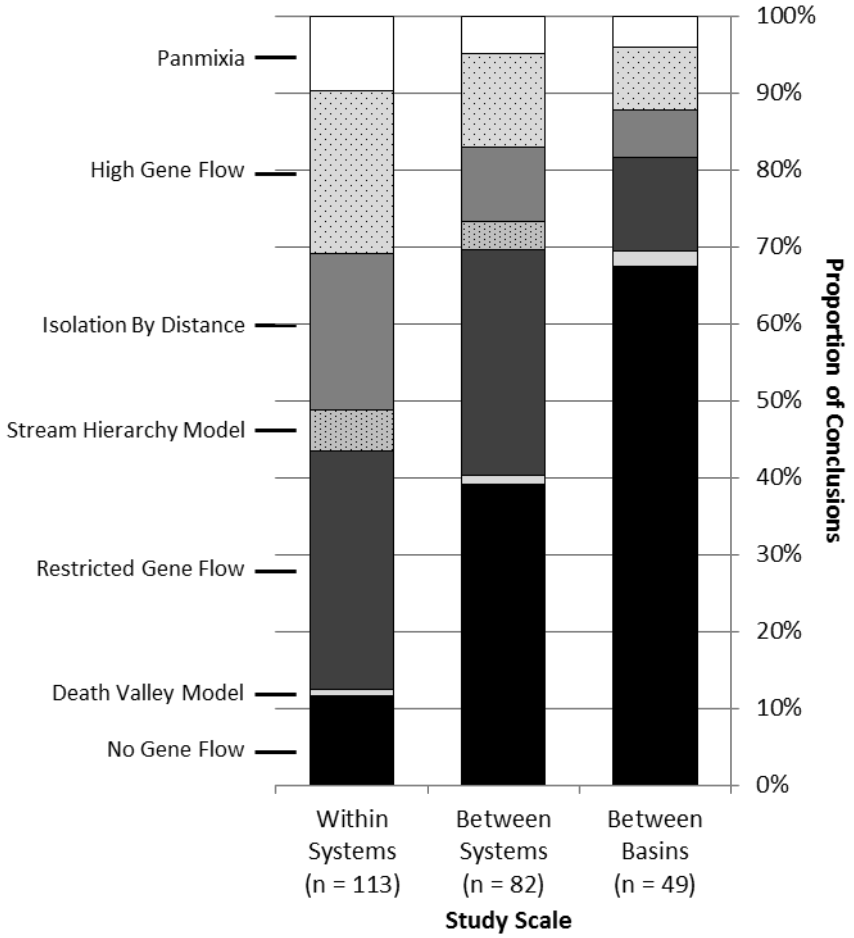

Figure 4. Percentage of studies of desert freshwater taxa that invoked each of seven connectivity models or gene flow descriptions, at three scales.

The prevalence of conclusions of high, restricted and no connectivity did not differ significantly among the three most-studied arid regions (the Australian arid zone, North American deserts and Mediterranean Basin drylands; $\chi^2 = 4.18 – 7.19$, d.f. = 4, $p = 0.13 – 0.38$). No significant differences in connectivity were found between species in the two most-studied habitat types (rivers and springs) at the two larger spatial scales ($\chi^2 = 2.54 – 4.81$, d.f. = 4, $p = 0.09 – 0.28$). In contrast, at the smallest scale, there were significantly more conclusions of low connectivity for species inhabiting spring systems than rivers ($\chi^2 = 9.71$, d.f. = 4, $p < 0.01$).

The three most-studied taxonomic groups (fish, crustaceans, molluscs; see Appendix 1.2) showed no significant differences in connectivity patterns from each other at any scale (χ^2 = 0.76 – 8.18, d.f. = 4, p = 0.09 – 0.94). However, species with higher dispersal abilities showed higher connectivity than moderately dispersing species at all three scales, while the moderate dispersers showed higher connectivity than the low dispersal ability species at all three scales (Fig. 5). These differences were significant at all three scales (χ^2 = 14.52 – 33.12, d.f. = 4, $p < 0.01$).

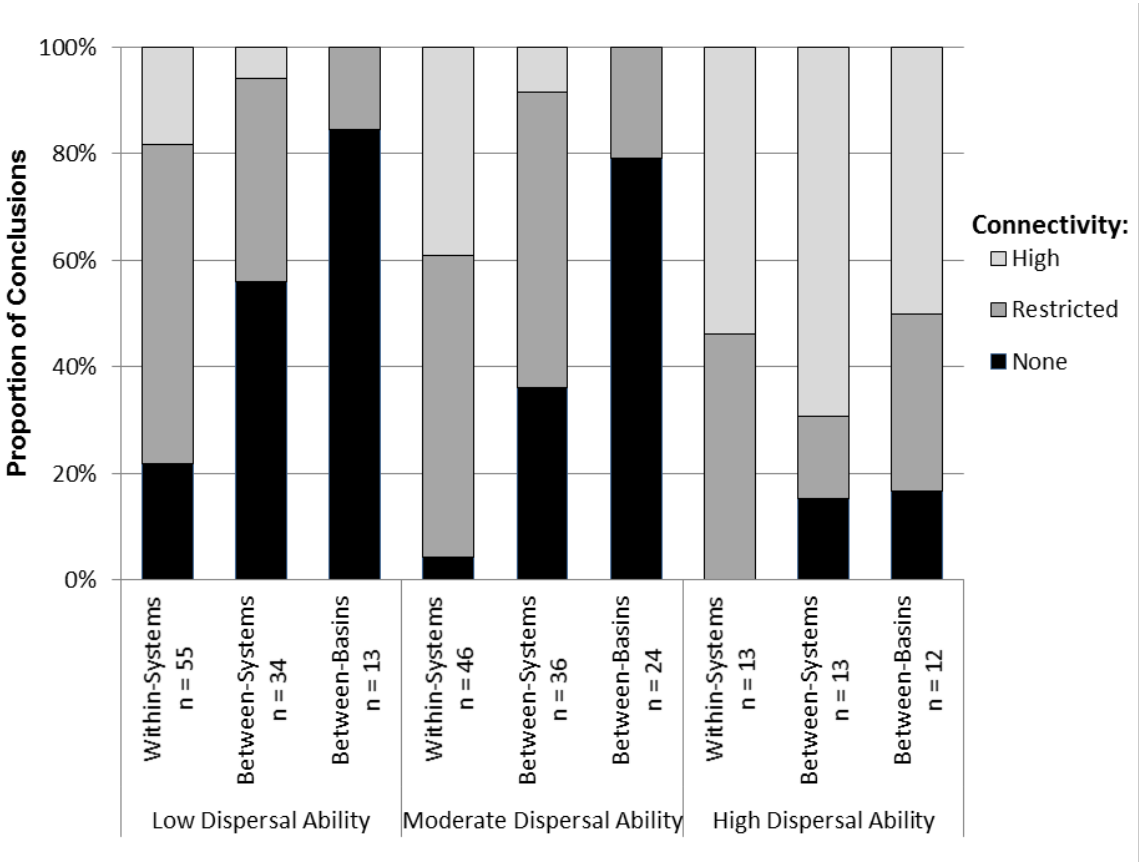

Figure 5. Proportion of studies of desert freshwater taxa that concluded three categories of connectivity, at three different scales, compared between species with three levels of dispersal ability.

Discussion

Our review of 133 studies of connectivity of aquatic fauna in desert freshwaters has shown that patterns of connectivity do not differ strongly between regions or taxa, or analytical methods used, but are strongly correlated with species' dispersal ability and habitat (spring or river). Connectivity declined at larger scales. Few studies tested existing connectivity models, and instead, most described the levels of gene flow or population structure. Here we

discuss these results, highlight future research opportunities and argue for analyses of connectivity in desert freshwaters that test pre-defined models of connectivity with probabilistic methods.

Advances in Molecular Population Biology

Since 1987, when the first study reviewed was published, molecular methods have advanced rapidly and many new data collection and analytical techniques have become accessible to ecologists. The general replacement of allozymes by microsatellites and sequence data typically provides greater power in connectivity studies; however their higher costs may mean that fewer loci can be included in a project. Some papers included very few loci indeed – almost 20% were based on just a single (usually mtDNA) locus. This is problematic because single-gene phylogenies reflect only the history of the gene (and in the case of mtDNA, the history of a maternal lineage), which may or may not mirror the population history (Edwards & Beerli 2000). In addition, a single gene might potentially be under selection or linked to genes under selection. Using multiple loci reduces these problems and can be considered independent tests of hypotheses, yielding higher sensitivity (Sunnucks 2000).

Technical advances have also allowed more marker classes to be used in studies, which has multiple benefits. The different processes governing the evolution of these markers means they can reflect different aspects of population biology and history, and different timescales. For example, a high level of subdivision was detected for the Australian spring snail *Fonscochlea accepta* in a study that used nine fast-evolving microsatellites (Worthington Wilmer *et al.* 2008), whereas no subdivision was detected when using a slower-evolving mtDNA marker (Murphy *et al.* 2010). Including multiple marker classes, notably uniparental as well as biparental, and sequence-based as well as frequency-based, will also allow a stronger understanding of historical and contemporary connectivity patterns.

While recent connectivity studies have utilised a range of modern analytical methods, most connectivity research has been based on deterministic models, especially F_{ST}. F_{ST} is a measure of the degree of difference between genetic samples (Wright 1951). The original model of F_{ST} was based on a network of panmictic sub-populations of the same constant size, connected by constant migration in migration-drift equilibrium, with no mutation and

no selection Under these conditions, gene flow is proportional to the inverse of F_{ST} and is unaffected by the geographic distance between populations (Wright 1951; Neigel 2002). The major assumptions of F_{ST} are often violated in natural systems (Marko & Hart 2011), and would seem to be especially unlikely to hold in the extreme and dynamic environments of desert freshwaters. In this environment, frequent changes in population sizes and connectivity, and accumulation of mutations in isolated populations, are expected to reduce the likelihood of the establishment of genetic equilibria.

While we found a strong shift over time towards methods that estimate gene flow with probabilistic models, some recent studies continue to use deterministic models. Unexpectedly however, we found no significant difference between the conclusions reached via the two methodologies. This surprising result has been found elsewhere, with F_{ST} shown to be robust to violation of assumptions and having a strong empirical track record (Neigel 2002; Whitlock 2011). Nevertheless, there is a strong chance that deterministic models will be incorrect in some studies, and where incorrect inferences are made, these will assume that gene flow plays a greater role in preventing divergence than is the case (Marko & Hart 2011). This can have negative effects on conservation management. For example, if resources are allocated to preserving wrongly-inferred gene flow, then conservation resources are wasted on neutral or even negative outcomes (e.g. outbreeding depression).

Fortunately, advanced genetic analysis methodologies, including coalescence, assignment methods and approximate Bayesian computation, mean that it is now unnecessary to make such assumptions, and allow more realistic modelling of complex connectivity scenarios (Marko & Hart 2011). These probabilistic models also offer other benefits, including for conservation. For example, estimates of divergence times can be used to determine whether the cause of divergence between populations is natural or anthropogenic, a key factor when deciding how to manage connectivity (Crandall *et al.* 2000). They also offer better estimates of levels of gene flow and a quantitative approach to studying connectivity, which allows optimisation of conservation management.

Modern approaches also offer a range of methods to test different connectivity models. We found that these models were under-utilised: very few papers considered a range of connectivity models, and many did not explicitly mention any model. While some studies

did not focus on connectivity, many that did ignored existing landscape connectivity models, even when they had the data to test them. Of those that did test models, most tested only Isolation by Distance and/or panmixia as null hypotheses, which in many cases are unlikely to reflect the true dispersal scenarios for aquatic organisms in deserts. Because models exist to aid understanding of systems, and connectivity modelling is vital for management and conservation of threatened species and communities (Vrijenhoek 1998; McRae *et al.* 2008; Ferrarini 2013), the extensive under-utilisation of connectivity models represents substantial missed opportunities.

Biases in Study Locations and Taxa

We found strong biases in the geographic locations and taxa included in reviewed studies. The bias we detected towards studying in developed countries and "close to home" has been noted in many fields, and is a major issue in field-based biology research (Pyšek *et al.* 2008). Here, it restricts our understanding of connectivity in desert freshwaters. Studies in the deserts of Africa, Asia and South America should be prioritised, and collaboration with local researchers required, as suggested in regard to phylogeographic studies by Beheregaray (2008).

The results of our literature search indicate that among those groups that were well-studied, there was a bias towards larger taxa, along with a focus on endangered fish taxa (especially among the North American and Iberian studies). Given the globally threatened nature of freshwaters, and limited research resources, studies should attempt to consider multiple species, ideally representing a broad sampling of relevant life history traits, in order to best inform management.

Drivers of Connectivity Patterns in Desert Freshwaters

While several connectivity models applied to freshwater systems explicitly consider geomorphology, principally drainage patterns and topography, hydrology is rarely incorporated. This may be because flows are generally constant and therefore have little effect on connectivity in the relatively well-studied mesic regions. In contrast, hydrology is extremely variable in most arid regions (Van Etten 2009), and likely has a greater effect than geomorphology in determining the connectivity patterns of many desert freshwater species (Sheldon *et al.* 2010).

Hydrology can affect connectivity in a number of ways. Higher frequency, larger volume and greater duration flows are all likely to increase connectivity. Climate change and anthropogenic disturbance scenarios largely predict flow will decrease and become more variable, leading to greater fragmentation and isolation (Woodward *et al.* 2010; Jaeger *et al.* 2014). However, few studies have been able to model these scenarios with respect to connectivity in desert freshwaters, and none of the studies included here did. Isolation by Resistance models offer an opportunity to explore the effects of hydrology: under a circuit-theory approach, lateral hydrological connections during flood events predicted the connectivity patterns of aquatic invertebrate communities in arid Western Australia (Morán-Ordóñez *et al.* 2015). Hydrology is a vital component of connectivity in desert freshwaters, and requires greater consideration in future studies.

Species' ecology is another major driver of desert freshwater connectivity patterns. While we found no significant differences in connectivity patterns among the three most-studied taxonomic groups (fish, crustaceans and molluscs), connectivity was significantly different between species with different dispersal abilities. Taxa with high dispersal ability showed high connectivity at all three scales, as expected, while some species with low dispersal ability showed no evidence of connectivity even at the smallest scale. While the overall pattern of decreasing connectivity at larger scales (Fig. 4) may seem an obvious result, this is not necessarily the case. Several studies found no change in connectivity across scales (e.g. Bostock *et al.* 2006; Stutz *et al.* 2010), because connectivity is dependent on the spatial scale relative to a species' dispersal ability. Accordingly, connectivity studies of ecological communities are increasingly analysed according to groups of taxa organised by dispersal mode or ability (Morán-Ordóñez *et al*. 2015; Phillipsen *et al.* 2015).

Many species showed less connectivity than expected given their dispersal ability, and while this is expected to be largely due to a lack of hydrological connectivity, there may be other reasons. One is selection pressure against dispersal (Maes *et al.* 2013), given the often low chance of finding a better habitat in arid environments. While most studies considered their taxon's dispersal ability, some ignored dispersal altogether and considered only geomorphology and/or hydrology as drivers of connectivity. Researchers need to incorporate dispersal and other life history traits when comparing population connectivity.

Researchers should also consider that different connectivity models may be appropriate at different scales, locations or habitat types. Dispersal traits are not necessarily identical for all members of a species: they may vary among locally-adapted populations, and according to local environmental differences (Baguette & Van Dyck 2007; Maes *et al.* 2013). Several studies found differences in connectivity patterns in different parts of a species' range, although this was always attributed to differences in structural connectivity (e.g. Carini & Hughes 2004; Murphy & Austin 2004; Huey *et al.* 2006). However, species may also be locally-adapted to different habitats. At the within-system scale, we observed significantly lower levels of connectivity in spring habitats than in rivers, and even no connectivity between springs separated by only a few hundred metres (e.g. Murphy *et al.* 2010). However, rather than resulting simply from limited hydrological connections, this pattern may reflect locally endemic species that are strongly dispersal-limited. Such a trait may be particularly useful in arid environments, where dispersal from permanent habitats, like springs, is highly likely to result in mortality (Maes *et al.* 2013). In contrast, behavioural experiments have shown the opposite for one desert dweller. Desert gobies, an arid Australian fish, from isolated spring-dwelling populations were often more dispersive than those from more-connected river systems (Mossop *et al.* 2017), which may be a mechanism for maintaining connectivity. If dispersal success does differ between populations (e.g. between those in temporary and permanent habitats), then connectivity patterns may differ (Berendonk & Bonsall 2002), and while this was not found in any of the studies reviewed, it should be considered.

The spatial and temporal transience of many populations of desert freshwater species adds additional complexity to connectivity studies. Many taxa exist in metapopulations, with subpopulations establishing during wetter times and becoming extinct when their habitat dries (e.g. Huey *et al.* 2011b). Such regular local extinctions and recolonisation events may erase the genetic signatures of the drainage pattern, masking true connectivity patterns. When colonisation events have occurred recently, equilibrium between genetic divergence and dispersal may not have been reached, meaning the assumptions of traditional genetic analyses are likely to be violated (Woods *et al.* 2010). Researchers should identify systems where a metapopulation structure is likely, and ensure that these processes are considered when studying desert freshwater connectivity.

Conservation Implications of Desert Freshwater Connectivity Studies

Understanding connectivity is vital for the conservation of desert freshwaters, especially given the major threats that climate change and anthropogenic impacts pose in arid regions (Dudgeon *et al.* 2006). For example, information on hydrological connectivity is useful for identifying refugia that allow multiple taxa to persist through drought periods. These should be priority sites for conservation management and protection, as these habitats facilitate the persistence of the three recognized levels of biodiversity (genetic, species and ecosystem), especially under adverse environmental conditions (Sheldon *et al.* 2002; Sheldon *et al.* 2010; Davis *et al.* 2013; Costelloe & Russell 2014; Jaeger *et al.* 2014). However, understanding the ecology of specific taxa is also important for management. Phillipsen *et al*. (2015) analysed three insect species with different dispersal abilities in North American desert streams and noted that climate change would affect each species differently. As such, each requires a different conservation management approach, and this is likely to be the case for many co-existing desert freshwater species.

While reporting connectivity patterns is clearly useful, incorporating connectivity models into studies is essential for conserving biodiversity and managing aquatic ecosystems (Hughes *et al.* 2013). Indeed, some models were built specifically to guide management. The Stream Hierarchy Model was developed for threatened North American desert fishes, and advocates management that maintains natural connectivity patterns and levels in order to maintain populations and their genetic diversity (Meffe & Vrijenhoek 1988). Because many desert freshwater species exist as metapopulations in transient habitats, there are often no habitats that require constant protection. Instead, protection of the processes driving connectivity is required, especially hydrological connectivity. In contrast, species that conform to the Death Valley Model exist as genetically-distinct, isolated populations, and should be maintained as such (Meffe & Vrijenhoek 1988). This model is especially applicable to short-range endemic species, such as the spring amphipods of central Australia, and their conservation requires both protection of their typically small habitats and prevention of any artificial gene flow (Murphy *et al.* 2013). On the other hand, panmictic species, such as those able to disperse widely via temporary connections during floods, may require protection of only a small number of key habitats (Moritz 1999). Connectivity models can inform a range of management decisions, including how limited conservation

resources are allocated, which aquatic habitats are prioritised for greatest protection, and the optimal management actions under a given set of goals and constraints.

Conclusions

How populations persist is a question that has fascinated scientists for decades (Mari *et al.* 2014). Answering this question is critical for the threatened aquatic ecosystems of the world's deserts. Persistence in these ecosystems often relies on connectivity, and we have shown that, regardless of location or taxonomic classification, this is driven by a species' ecology and the hydrology of its habitat. Untangling the effects of these drivers is complex, and we have conducted the first 'health-check' of research in this field. We advocate obtaining DNA sequence data from mitochondrial and multiple nuclear markers, and applying an integrated range of approaches, including coalescent analyses and approximate Bayesian computations, to estimate gene flow and other key population parameters at different temporal and spatial scales. These estimates can be used to test a range of explicit models of connectivity. Such an approach will give greater power and accuracy, and minimise the number of assumptions. We draw attention to the advantages of greater utilisation of existing connectivity models, and extending these to more realistically address questions in specific regions (Morán-Ordóñez *et al.* 2015). Finally, studies are urgently needed on the desert freshwaters of Africa, Asia and South America. These systems have received little research attention, yet face the same threats as other desert freshwaters, which are among the most threatened ecosystems on the planet.

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Chapter Three

From Dry to Wet and Back Again: The Environment and Ecology of the Lake Eyre Basin's Rivers

Introduction

Arid and semi-arid regions cover more than 30% of the world's surface area and are especially prevalent across Australia, where they account for around 70% of the continent (Peel *et al.* 2007). The Australian arid zone, defined by an annual rainfall less than 500 mm and a potential evaporation rate exceeding this (Kingsford 2006), has a climate characterised by low and highly variable rainfall, and high diurnal temperatures (Hobbs *et al.* 1998). The region has a 'boom and bust' ecology, with relatively long droughts interrupted by brief and unpredictable rainfall events that temporarily transform the desert into productive and diverse communities (Bunn *et al.* 2006b).

The Lake Eyre Basin (LEB) is located in arid central Australia and covers an area of more than 1.2 million km^2 (one-seventh of the continent; Fig. 1). It includes large parts of Queensland, South Australia and the Northern Territory, as well as a small part of western New South Wales. A lack of uplift and continued erosion have resulted in a flat, low landscape, with the vast majority of the LEB less than 200 m above sea level, and Lake Eyre itself 15.2 m below sea level (Twidale & Campbell 2005). Areas of significant relief are found only on the periphery of the Basin, including the MacDonnell and Flinders ranges. Despite the aridity, most of the LEB is vegetated, predominantly by tussocklands and herbfields (Martin 2006; Byrne *et al.* 2008). Deserts, including the Simpson, Strzelecki, Tirari and Sturt Stony, cover around a fifth of the LEB (Geoscience Australia 1994).

The LEB incorporates a number of river catchments surrounding the ephemeral Lake Eyre, and is the world's largest endorheic (internally-draining) basin. There are no contemporary hydrological connections to adjacent basins, which include a number of smaller tropical basins to the north and north-east that drain to the Gulf of Carpentaria and Coral Sea; and the Murray-Darling Basin to the east, and Lake Torrens Basin to the south, which terminate

in the Southern Ocean (Fig. 1). The endorheic Bulloo Basin is immediately east of the LEB and was historically connected, but became isolated at an unknown time (Wager & Unmack 2000). To the west, the large Western Plateau drainage division contains no major river systems (Unmack 2001a). Within the LEB, long-term aquatic habitats are restricted to groundwater-fed springs and a network of ephemeral rivers, which exist for most of the year as series of isolated waterholes (Fig. 2; McMahon *et al.* 2008a). When rain does fall, these channels transport flows over great distances towards Lake Eyre, although much of the water is lost as the floods spread out across vast floodplains (Puckridge *et al.* 1998).

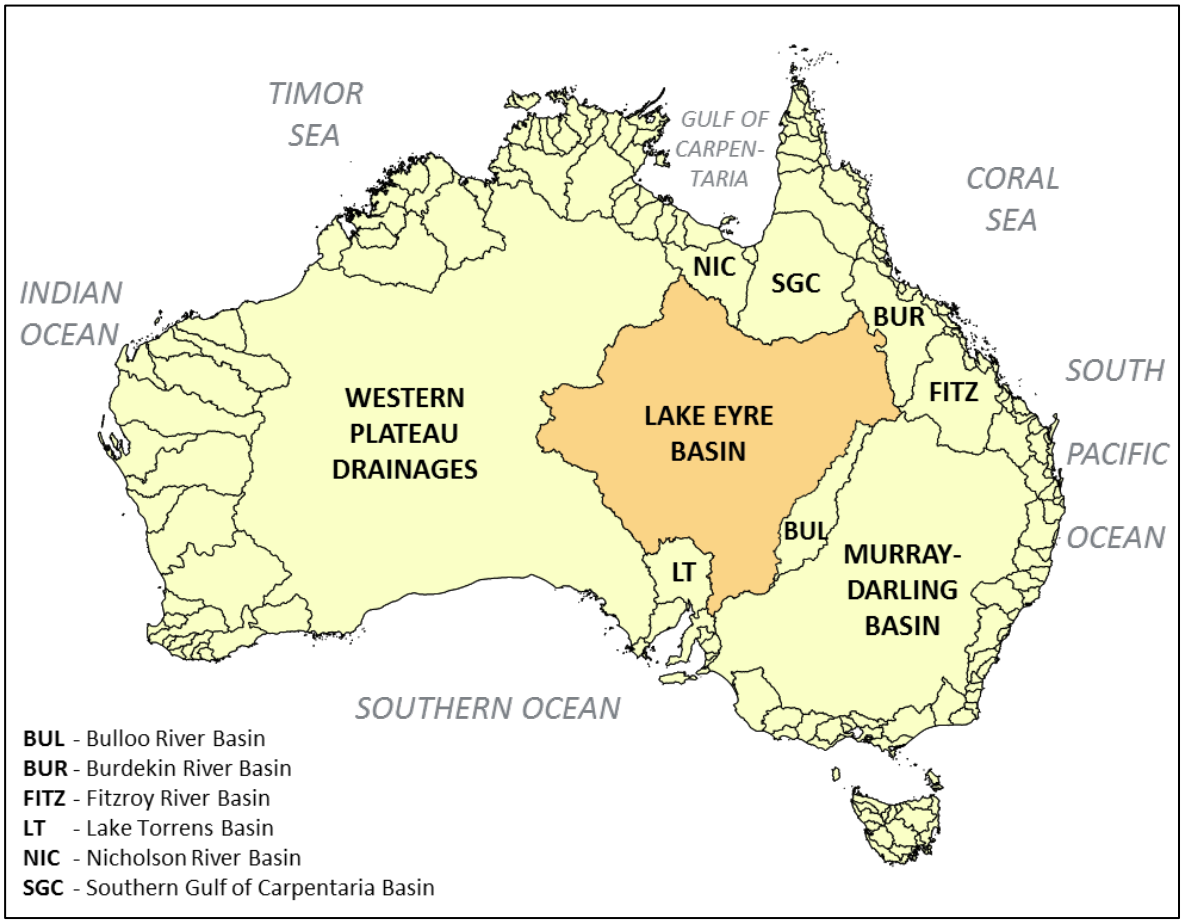


Figure 1. Map of the river basins of Australia, showing the location of the Lake Eyre Basin and adjacent drainages.

The rivers of the LEB remain unregulated and have experienced relatively minimal anthropogenic disturbance compared with other Australian rivers and those in arid regions globally (Costelloe & Russell 2014). As with most arid regions, many of the LEB's aquatic habitats are inhabited by fishes, despite the extreme aridity and minimal permanent water. Thirty-three native fish species are found in the region, and hydrological connectivity is

thought to be vital for the long-term survival of their populations (Wager & Unmack 2000; Sheldon *et al.* 2010). The persistence of these fishes in this dynamic landscape is impressive, and offers an opportunity to explore how populations utilise connectivity to persist in extreme environments.

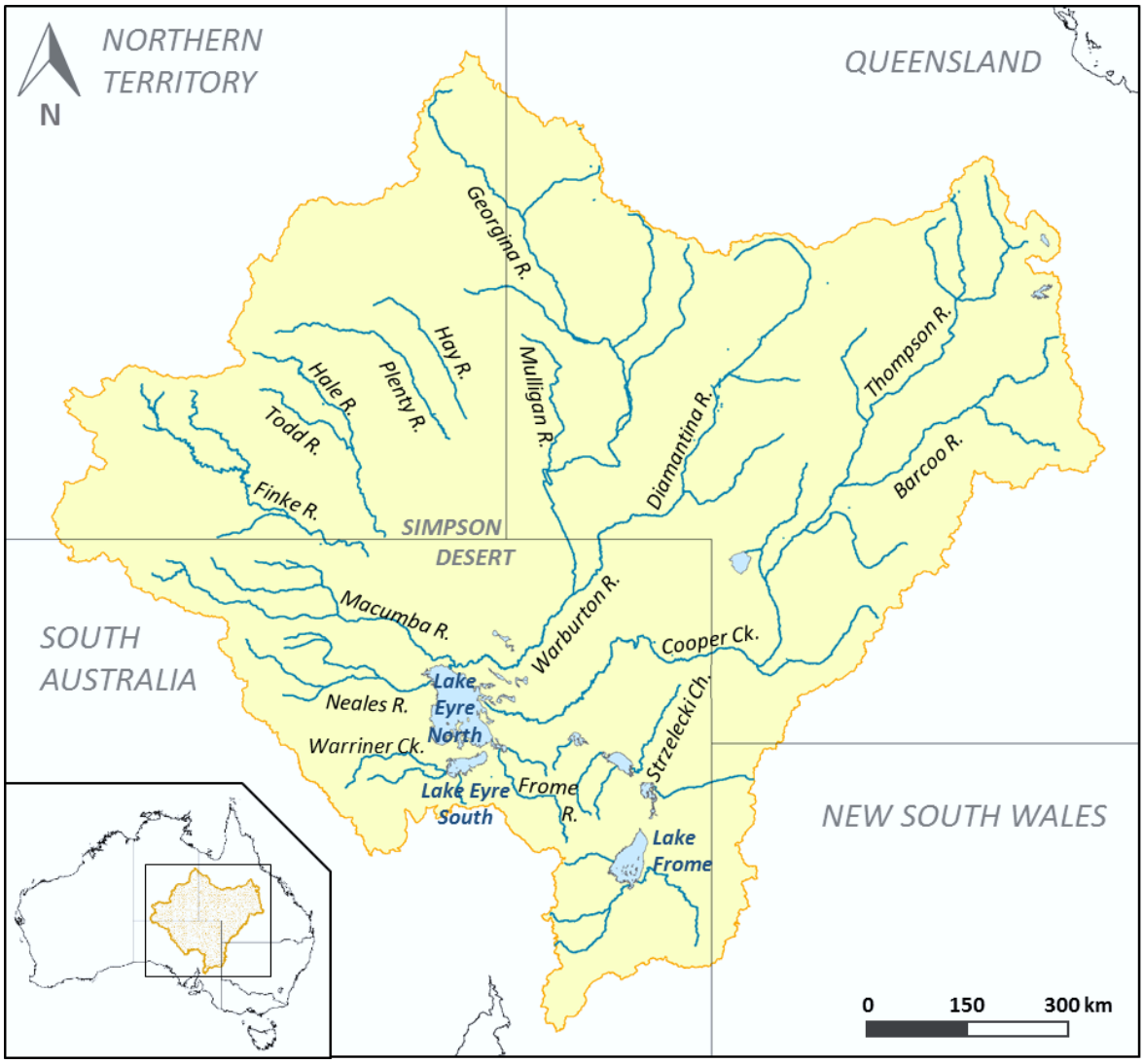


Figure 2. Map of the Lake Eyre Basin, showing the courses of its major river systems and main tributaries, and the locations of large lakes and the Simpson Desert.

In order to understand the environment these fish inhabit, the history, waterbodies, hydrology and ecology of the LEB are reviewed here, with a strong focus on its aquatic habitats. This provides a platform upon which the fishes inhabiting this region are explored in the following chapter, considering their biogeography and ecology, including life history strategies that allow persistence in the LEB.

The History of the Lake Eyre Basin

The LEB was formed through tectonic subsidence in what is now northern South Australia, during the late Palaeocene ~60–56 Mya (million years ago; Alley 1998). Other geographic events, such as orogeny and volcanism, have not significantly affected the LEB since this time. In fact, the region has remained geologically stable since the uplift of the Flinders Ranges in the south-east LEB 65 Mya (Veevers 1991). While dolphin fossils dated to the Late Oligocene (24–28 Mya) indicate marine connections to the sea in the past (Fitzgerald 2004), the LEB has been endorheic since (Alley 1998). Here, the environmental changes in the LEB since the Miocene are reviewed, especially the impacts on freshwater systems.

The Australian continent has been isolated for 45 million years, following its disconnection from Antarctica and subsequent northward drift. This drift, as well as changes to global climate, have driven massive changes in the continent's environment, perhaps most apparent in central Australia, including the LEB. Climatic changes have seen this region transition from humid rainforest to one of the driest deserts on Earth. While the aridification process likely started following the continent's isolation and the formation of the Antarctic Circumpolar Current, the most extreme changes began in the Miocene (Byrne *et al.* 2008).

At the start of the Miocene, 23 Mya, central Australia had a warm, wet climate and was dominated by rainforest (Byrne *et al.* 2008). There were extensive, shallow lakes, including Lake Eyre and a number of others stretching southward, which supported a range of aquatic species, notably flamingos, crocodiles and lungfish (Martin 2006). By the mid-Miocene however, rising temperatures led to greater evaporation and a more arid climate, causing the contraction of rainforest to sheltered habitats and the expansion of dry, open woodlands and shrublands (Martin 2006). The mid-Miocene saw the end of regular flows in the drainages that occur immediately west of the LEB, and the loss of significant drainages in central Australia (Van de Graaff *et al.* 1977; Quilty 1994).

While aridity continued to increase to the end of the Miocene, the start of the Pliocene 5–3 Mya saw a brief shift to a wetter climate, and a corresponding expansion of lakes and other aquatic habitat (Fujioka & Chappell 2010). The subsequent return to aridity was accompanied by the appearance of the first desert landscapes, with stony desert formation beginning ~4–3 Mya, (Fujioka *et al.* 2005). During the aridification of the LEB in the

Miocene and Pliocene, the region's biota underwent significant change, including the diversification and radiation of many plant and animal lineages, both terrestrial and aquatic (Byrne *et al.* 2008).

During the Pleistocene, central Australia was subject to the same climatic cycles as the rest of the world, but manifested in a different way to the northern hemisphere. No glaciation occurred in central Australia during glacial periods; instead, these periods were characterised by extensive aridity (Martin 2006; Fujioka & Chappell 2010). Interglacial periods were milder, but still dry. The climatic cycles resulted in vegetation shifts, with loss of vegetation in glacial periods resulting in widespread erosion and the further development of desert landscapes (Martin 2006; Byrne *et al.* 2008). The first sand dunefields appeared in central Australia during this time (Fujioka & Chappell 2010). Although dating has not been confirmed for all dunefields, the first dunes in the Simpson Desert (the largest dunefield in the LEB) formed ~1 Mya (Fujioka *et al.* 2009). Dunefields expanded during the later Pleistocene, especially during glacial periods, leading to the isolation of a number of rivers in the north-west of the LEB from Lake Eyre (Craddock *et al.* 2010).

The Pleistocene also saw the decline of extensive freshwater lake systems in central Australia, with a transition from permanent to ephemeral lakes (playas) occurring from ~900 kya (thousand years ago; Chen & Barton 1991). By 500 kya, many lakes had become saline playas, although transition timing would have depended on local hydrological conditions, and reversals would have occurred, especially during interglacial periods (Fujioka & Chappell 2010). The increasing aridity also saw the demise of Australia's largest mega-lake system, Lake Dieri, which extended from Lake Eyre to Lake Frome, with the connection to Lake Eyre severed around ~50 kya (Cohen *et al.* 2011). This decline was largely driven by a reduction in precipitation, and it is likely that rivers of the LEB may also have become ephemeral during glacial periods (Nanson *et al.* 2008). The strong climatic oscillations of the Pleistocene resulted in repeated changes to the permanence of LEB waterbodies, with Lake Eyre itself reverting to a perennial system at ~125 kya, 80 kya, 65 kya and 40 kya (Magee 1997). Towards the end of the Pleistocene, the continued decline of large waterbodies led to the loss of many aquatic species, including lungfish, crocodiles and flamingos (Hope 1982).

The Last Glacial Maximum (LGM; ~21 kya) was a time of extreme aridity and sand dune-building activity in central Australia (Hesse & Simpson 2006; Byrne *et al.* 2008). The interglacial period after the LGM was characterised by dune stabilisation by vegetation in a wetter climate (Luly 2001). This was especially prevalent in the south of the LEB, during a humid phase ~17–15 kya (Fitzsimmons *et al.* 2013). During this time, and extending into the Holocene, there is evidence of increased river flows in the north-eastern LEB, potentially due to larger or more frequent monsoonal rains (Nanson *et al.* 2008). However, the climate has become progressively more arid since then, and appears to continue a trend of each interglacial being more arid than the one before, although not as arid as the previous glacial (Martin 2006; Fitzsimmons *et al.* 2013). This increasing aridity has had a strong impact on the environment of the LEB and its biota, especially aquatic biodiversity (Byrne *et al.* 2008).

The Climate of the Lake Eyre Basin

Central Australia is one of the world's driest landscapes, with long periods of drought interrupted by bursts of intense rainfall (Morton *et al.* 2011). The region around Lake Eyre receives an average of just 110 mm of rainfall per year, with higher rainfall in the north and periphery of the Basin (Fig. 3A). The north-eastern LEB receives the greatest rainfall, averaging up to 500 mm per year, largely from irregular tropical cyclones (McMahon *et al.* 2008a). Rainfall is also higher around the small mountain ranges on the edge of the LEB, including in the south-west (Flinders Ranges) and north-west (MacDonnell Ranges). Mean annual runoff across the LEB is estimated to be around 4 km^3, the lowest of any major drainage basin in the world (Kotwicki & Isdale 1991; Kotwicki & Allan 1998).

Rainfall is extremely variable and unpredictable, with most of the LEB (i.e. the area north of latitude 27°S) being the most variable in the world and comparable to the Somali, Namib-Kalahari and Thar deserts (Van Etten 2009). In contrast, rainfall of the southern quarter of the LEB is significantly less variable, and comparable to that in deserts in North America and northern Africa (Van Etten 2009). The southern LEB generally receives rain from predictable cold fronts during the winter months, while the northern LEB's rainfall is largely derived from tropical cyclones during summer (Martin 2006). The north-eastern part of the Basin experiences irregular monsoonal rains, which are most common during La Niña phases of the El Niño Southern Oscillation phenomenon (McMahon *et al.* 2008a).

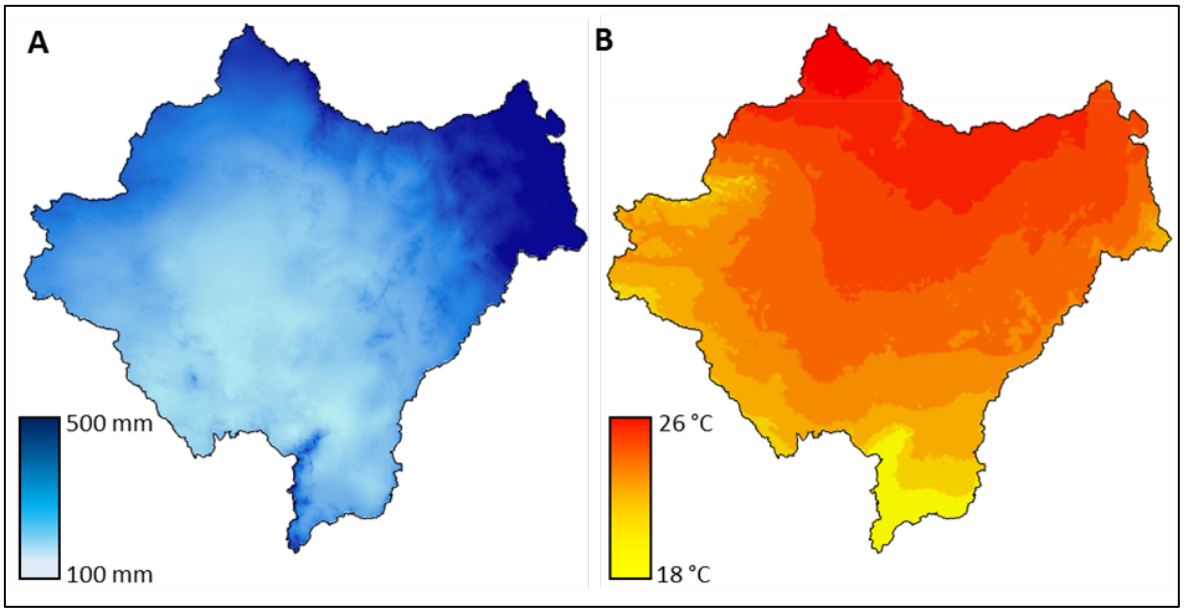


Figure 3. Mean annual A) rainfall and B) daily mean temperatures across the Lake Eyre Basin. Climate data source: Bureau of Meteorology (BOM; available at: http://www.bom.gov.au).

The region experiences high temperatures year round, with average daily mean temperatures ranging from 18–26 °C (Fig. 3B). Temperatures are higher during the summer months (maximum daily temperatures average 35-39 °C), and may reach 50 °C rarely (Wager & Unmack 2000). The highest temperature recorded in the LEB, and indeed Australia, was 50.7 °C on 2 January 1960 at Oodnadatta in northern South Australia (BOM 2017). Temperatures are lower during winter (maximum daily temperatures average 16-24 °C), and overnight lows may occasionally drop below freezing (Wager & Unmack 2000). Temperatures are warmer in the north, with more seasonal variation occurring further south. Potential evaporation rates are very high, and range from 2400 to 3600 mm per year, far greater than the amount of precipitation (Kotwicki & Allan 1998).

The Waterbodies of the Lake Eyre Basin

A range of waterbodies are present in the LEB, from large ephemeral lakes to small permanent springs, distinguished by their hydrology and geomorphology (Davis *et al.* 2013). These have been classified by studies that cover parts of the LEB (e.g. Duguid *et al.* 2005; Fensham *et al.* 2011) or the entire Australian arid zone (e.g. Davis *et al.* 2013). Here, permanent or long-lasting waterbodies that provide long-term habitat for fishes, and ephemeral waterbodies that provide hydrological connections between permanent waterbodies, are focused on. The former include riverine waterholes and springs, while the latter include ephemeral rivers and lakes. Other waterbodies are present, but unlikely to be utilised by fishes, either because they are hydrologically isolated (e.g. rockholes) or are only briefly filled (e.g. claypans; Davis *et al*. 2013). For this study, spring habitats are largely excluded (but see below). Stream-based waterbodies, and ephemeral connections between them, are focused on, due to their role as the primary habitat for fishes in the LEB.

The LEB is divided into a series of catchments or drainages, each flowing (or historically flowing) towards Lake Eyre. Because of the low-relief of the LEB, individual catchments are vast, and are often separated only by low-lying hills (Wager & Unmack 2000). Rivers are generally of very low-gradient, meaning they flow slowly, and spread laterally, when they do flow (McMahon *et al.* 2008b). While most LEB rivers flow (at least in some sections) for some time each year, for the majority of the time the rivers exist as a series of waterholes separated by dry channels – there are no permanently flowing rivers (McMahon *et al.* 2008b). These catchments are largely fed by rain events with some groundwater contributions, but no snowmelt or other regular or seasonal water sources, in contrast with many other deserts globally. Groundwater contributions are largely derived from the Great Artesian Basin (GAB), the world's deepest and largest artesian basin, which covers 1.7 million km^2 and underlies much of the LEB, except the north-west and far south (Habermehl 1980). While the extent to which these contributions affect LEB rivers is little known (but see Fensham *et al.* 2011), it is likely to be minimal, and indeed more water probably flows from rivers into the GAB (recharging it) than the converse (McMahon *et al.* 2008b).

Springs

The LEB contains eleven "supergroups" of springs, totalling 600 outlets, each feeding up to 400 individual springs (Wager & Unmack, 2000). These springs typically form mounds (Fig. 4), although a few form artesian springs or bogs. Of the supergroups, four contain fish – the Dalhousie, Edgbaston, Elizabeth and Lake Eyre Springs. While some of these springs may connect to river systems during rain events, they are generally disconnected from the main rivers of the LEB (Mossop *et al.* 2015). There are a few small, individual springs located on watercourses, providing additional groundwater inputs to rivers on a localised basis. Apart from these exceptions, which function essentially as permanent waterholes from a fish's perspective, springs have been excluded from this study due to their isolation, permanence and other differences to the river systems of the LEB. A number of anthropogenic waterbodies also exist within the LEB, including bores, drains, dams and impoundments, which may contain water permanently (depending on the source), as well as fish (Wager & Unmack, 2000). These have also been excluded from this study, except where they occur on a watercourse.

Figure 4. Blanche Cup, one of several mound springs in the South-West Creeks area of the Lake Eyre Basin. This spring sits around 10 metres above the surrounding plain.

River Systems

The LEB is divided into several major drainages, many of which are extremely old, with the Finke River particularly ancient and thought to have flowed through central Australia for up to 350 My (Pickup *et al.* 1988). The largest river systems include the Georgina-Diamantina River, Cooper Creek, Frome River and Neales River, which feed into Lake Eyre North (Fig. 2; Table 1). A series of smaller creeks feed Lake Eyre South, including Margaret, Warriner, Screechowl and Finniss creeks. Currently, only 68% of the LEB contributes water to Lake Eyre (McMahon *et al.* 2008b). The rest of the Basin is disconnected from the Lake, including large areas of desert with no river networks (McMahon *et al.* 2008b). The formation of the Simpson Desert severed the connection between the Lake and several rivers to the north-west, including the Hay, Plenty, Hale, Todd and Finke rivers (Unmack 2001b), referred to here as the 'Desert Rivers'. Additionally, the decline of the extensive freshwater lake systems in the Pleistocene led to the disconnection of the Lake Frome catchment, in the far south of the LEB, although a connection to Cooper Creek means this catchment may receive some water from the main LEB during very large flood events (DeVogel *et al.* 2004).

Table 1. The drainages of the Lake Eyre Basin and their major rivers that contain fish (Wager & Unmack 2000), the number of permanent waterholes in each river (Fensham *et al.* 2011), how often their flows reach Lake Eyre (Kotwicki & Isdale 1991), and their relative within-drainage hydrological connectivity.

Drainage	River	Permanent Waterholes	Reaches Lake Eyre	Hydrological Connectivity
Cooper Creek	Cooper Creek	~200	1 in 6+ years	High
Georgina-Diamantina	Diamantina River	~25	1 in 2 years	High
	Georgina River	22		
	Macumba River	0	1 in 10 years	Low
Desert Rivers	Hay River	~0?	Never	Low
	Plenty River	~0?		
	Todd River	0		
	Finke River	7		
Western Rivers	Neales River	1	1 in 5 years	Low
South-West Creeks	Warriner Creek	2	1 in 5 years?	Low
	Margaret Creek	?		
Frome Drainage	Frome River	0	?	Low

The Channel Country rivers, including the Georgina-Diamantina and Cooper, largely lie in Queensland (Fig. 2). These rivers are long, with wide anabranching channels (i.e. sections of the river divert from the main channel and re-join downstream) especially in the mid-lower reaches, and flow relatively frequently due to their location in the least-arid part of the Basin.

The Georgina-Diamantina River system contributes around 60% of Lake Eyre's inflow, and is the largest river system in central Australia (Nanson *et al.* 1998; McMahon *et al.* 2008b). The Georgina River (length to Lake Eyre 1,130 km) starts in the far north of the LEB, and is fed by a number of tributaries (Templeton, Sandover, Burke, Hamilton and Mulligan rivers), before merging with the Diamantina (720 km long) at Goyder Lagoon to form the Warburton River, which is met by the Macumba River and its tributaries (Alberga River, Hamilton Creek) just north of Lake Eyre. Because of its reach into northern Australia, the Georgina-Diamantina is fed regularly by tropical cyclones and monsoonal rains, although the Diamantina contributes the majority of flow (in terms of frequency and volume of contributions; Kotwicki 1986). The Warburton River is thought to contribute inflow to Lake Eyre approximately once every two years (Kotwicki 1986). As with other long river systems in the LEB, during flood events the majority of water (>75%) is lost during transmission through the fluvial system (referred to as 'transmission losses'), mostly due to evaporation (Hamilton *et al.* 2005).

Cooper Creek is south-west of the Georgina-Diamantina, and includes the Barcoo and Thompson Rivers as major tributaries. It is the longest of the LEB rivers, and the second-longest in Australia (after the Murray-Darling) at 1,300 km. The Cooper flows relatively frequently, and contributes around 15% of the Lake's inflow, which it is thought to reach approximately one in six years (Kotwicki 1986; Nanson *et al.* 1998). However, this may be an overestimate, as the Cooper has only flowed into the lake three times since the 1980s, in 1990, 2000 and 2010 (Justin Costelloe, *pers. comm.*). When in flood, the low gradient of the river allows the flow to move laterally, resulting in extremely wide channels that cover vast floodplains. During a large flood event in 1940, parts of the Creek were estimated to be 43 km wide ("Floods Only Bar to Rats" 1940). In its lower reaches, the Cooper is only separated from the Goyder Lagoon (Georgina-Diamantina) by 20–30 km of low-lying land, and could potentially be connected in massive flood events (Kotwicki 1986).

The remaining inflow to Lake Eyre is derived from smaller rivers to the south and west of the Lake, in South Australia, that are relatively poorly known. The largest, the Neales River (and its tributary, Peake Creek), rise to the west among elevated terrain (Fig. 5). The Neales flows for around 430 km to the Lake, which is reached approximately twice per decade (Kotwicki 1986). Several smaller creeks also run into Lake Eyre North from the west, including Douglas, Sunny, Cooinchina, Koorakarina and Anchor creeks. Because these creeks, and the Neales, are relatively short, they experience reduced transmission losses during flow events, and thus are more likely to reach Lake Eyre following smaller rainfall events than the Channel Country rivers (Kotwicki 1986). Large rainfall events of >50 mm (expected to occur less frequently than once per year) are required for full channel flow of these smaller watercourses, while smaller falls (15–20 mm) can cause brief (a few hours) flows (Kotwicki 1986). Frome River flows for 245 km from the south-east, with headwaters in the Flinders Ranges, and tributaries including Taylor, Mundy and Leigh creeks (Kotwicki 1986). Little is known about this river system.

Figure 5. A dry river channel in the Neales River, in the west of the Lake Eyre Basin, showing a sandy riverbed and persisting perennial vegetation.

Lake Eyre South is fed by a series of streams (here termed the South-West Creeks), the largest of which are Warriner and Margaret creeks, with lengths of 210 and 155 km respectively (Kotwicki 1986). Other creeks flow from the south, including Screechowl, Finniss, Stuart, Gregory, Alberrie and Nelly creeks, but there is very little information

available on these systems (Dale McNeil, *pers. comm.*). Despite their short lengths and small catchment areas, these creeks can fill Lake Eyre South rapidly following rain, and their floods are likely of similar magnitude to those flowing to Lake Eyre North (Kotwicki 1986).

To the north of Lake Eyre lie the Desert Rivers, which are separated from the Lake by the Simpson Desert, where they currently dissipate. There are three main drainages here, which all start in ranges along the north-western periphery of the LEB. The Hay and Plenty Rivers, which start in the Harts and Dulcie ranges (near the headwaters of the Sandover River, a tributary of the Georgina), run for ~400 km south-east, before dissipating in floodouts among sand dunes (Duguid 2011). Prior to the Simpson Desert expansion, these rivers were tributaries of the Georgina River (Craddock *et al.* 2010). Similarly, the Todd and Hale Rivers rise in the MacDonnell Ranges, and run south-east for 315 and 400 km respectively, before disappearing into the sand (Unmack 2001b). These rivers may confluence in exceptionally large flood events, and historically would have likely then joined the Finke (Craddock *et al.* 2010). The Todd demonstrates the variability of LEB rivers, as it experienced Australia's largest recorded flood, measured as a ratio of mean annual flood (McMahon 1985).

The Finke River (Fig. 6) also has its headwaters in the MacDonnell Ranges, and due to bisections through multiple parts of these ranges, the upper reaches are thought to pre-date the uplift of the MacDonnells 300–350 Mya (Pickup *et al.* 1988; Haines *et al.* 2001). The Finke flows south-east for 730 km, with major tributaries including Palmer and Hugh rivers, and Karinga, Lilla and Goyder creeks, before dissipating into floodouts in the Simpson Desert. Historically, the Finke was part of a larger drainage network that likely joined the Macumba River (Craddock *et al.* 2010). The Finke was the last of the Desert Rivers to be disconnected by the westward expansion of the Simpson Desert, but the timing of this event is unclear, with estimates of 10–20 kya (Unmack 2001b).

The Finke typically terminates at a large floodout just north of the South Australian border, the Finke Floodout Forest, where flows are blocked by Mt Wilyunpa (Duguid 2011). This floodout is reached approximately once per decade, with known events including floods in 1921, 1967, 1972, 1974, 1988, 1993, 2000, 2010 and 2015 (Angus Duguid, pers. comm.; Duguid 2013). After large flood events, a second floodout on Snake Creek can also be reached, including in 1967, 1971, 1974, 1993 and 2000, filling vast areas between the dunes and creating a series of long-lasting lakes (Duguid 2005). For example, after the 1967 flood,

these interdune corridors were filled to a depth of up to 6 m, which persisted for up to 19 months (Williams 1970). After the 2000 flood, these wetlands were filled to a depth of 9–10 m, had lengths greater than 12 km, and persisted for up to 30 months (Duguid *et al.* 2005).

Figure 6. A typical dry river channel in the Finke River drainage in the Lake Eyre Basin; wide and flat, and lined with *Eucalyptus* trees.

There is also a channel leading south from the Finke Floodout Forest into South Australia and terminating at a third floodout (Duguid *et al.* 2005). Extremely large flows have reached this in 1967, 1974 and 2000 (Williams 1970; Duguid *et al.* 2005). However, they have never been recorded to extend beyond this. As the 1974 flood is thought to be the largest since a flood around 850 years ago, it is likely that no flows have reached beyond this point for at least the last 850 years (Pickup 1991). However, it has been suggested that massive flood events (1 in 500–1,000 year event) could connect through to the Macumba River, and then to Lake Eyre (Duguid 2011). While there is no direct support for this, this could theoretically happen if flows did extend past the South Australian floodout. This floodout is within 10 km of the floodplain of the Dalhousie Springs system, which is itself around 80 km from the nearest part of the Macumba River catchment (Duguid *et al.* 2005). So, hydrological connections could occur through this area following a massive flood event in the Finke, although this would likely also require simultaneous heavy and widespread flooding in the Macumba catchment (Duguid 2011).

Kati Thanda - Lake Eyre

Lake Eyre, also called Kati Thanda – Lake Eyre, recognising the local Arabana name for the Lake, can cover 10,000 km^2 when full and is the 18th largest lake in the world (Leon & Cohen 2012). The lake sits in north-east South Australia, and its deepest part is 15.2 m below sea level – the lowest point on the Australian continent (Kotwicki 1986). The lake comprises two parts, Lake Eyre North (144 km long, 65 km wide) and Lake Eyre South (65 km wide, 24 km long), connected via the 15 km Goyder Channel. While historically the Lake was a permanent feature, it transitioned to a playa during the Pleistocene, and currently rarely fills. As such, it is normally dry; a vast desert of salt and clay, with a few saline pools (Wager & Unmack 2000; Habeck-Fardy & Nanson 2014). Lake Eyre was not known to fill until over 100 years after its discovery. In 1840, Edward John Eyre became the first European to see the lake when he reached the southern edge of Lake Eyre South (Kotwicki 1986). As the Lake was dry at the time, it was believed to never fill, and despite reports of the presence of water in 1869 and 1922, it was not until a large filling event in 1949 that this was confirmed (Kotwicki 1986). Lake Eyre is estimated to hold around 400 million tonnes of salt, which forms a crust when the Lake dries (Fig. 7) that can reach a depth of 460 mm and cover an area of over 2,500 km^2, floating atop a slush of sediment and groundwater (Bonython 1956; Dulhunty 1977).

Figure 7. A view of Lake Eyre South when dry, with the white salt crust clearly visible. Photo taken 15 September 2014, eight months after a small fill event in the lake.

Inflows to Lake Eyre occur on average every two years, although periods of up to seven years with no inflow have been observed. The mean average inflow has been estimated at 3.8 km^3, with a standard deviation of 6.2 km^3 (Kotwicki 1986). Larger inflows are more likely to occur during La Niña phases of the El Niño Southern Oscillation phenomenon (Kotwicki & Allan 1998; McMahon *et al.* 2008a). While inflow events are relatively common, they rarely lead to the Lake becoming 'full'. Minor fill events (inflow of up to 10 km^3) occur when large flow events occur in one of the main drainages, while major fill events (20–30 km^3) that cover more than half the lake area, result from large flow events in multiple drainages, which occur around once every ten to twenty years (Kotwicki & Allan 1998; Leon & Cohen 2012). Major fill events can take several months to complete, with the following 'drying up' process taking one to three years (Kotwicki 1986). Recent major fills of Lake Eyre North have occurred in 1949–1950, 1974–1977, 1984, 1989–1990, 1991–1993, 1997, 2000–2001, 2009–2011 and 2015–2016 (Kotwicki & Isdale 1991; Hope *et al.* 2004; McMahon *et al.* 2008b; Leigh *et al.* 2010; Backway 2014).

Lake Eyre South fills more frequently, due to its smaller size and the presence of more, and shorter, tributaries. These receive higher rainfall than rivers flowing to Lake Eyre North, and experience lower transmission losses. Lake Eyre South is known to have filled in 1938, 1955, 1963, 1968, 1974–1976, 1984, 1989, 1992, 1997, 2000, 2001, 2003, 2010–2011, 2012 and 2015–2016 (McMahon *et al.* 2008b; Backway 2014). The largest of these filling events was the 1974 flood, which resulted from inflows from all currently-connected LEB drainages following massive annual rainfalls in both the eastern (700 mm) and western (500 mm) parts of the LEB (Kotwicki 1986; Habeck-Fardy & Nanson 2014). These filled Lake Eyre North, which then overflowed into Lake Eyre South via the Goyder Channel. The 1984 fill event was much smaller, but both Lake Eyre North and South filled in just a few days following several days of heavy rainfall (180–360 mm) in the western drainages (Kotwicki 1986). Connections also occurred during the 1984 and 1989 floods when Lake Eyre South overflowed to Lake Eyre North (Kotwicki 1986; Habeck-Fardy & Nanson 2014). It is unclear if the lakes have connected since, although the larger fills of 2009–2011 and 2015–2016 may have permitted this.

Upon filling, the environment of Lake Eyre changes drastically. Topographically, the bottom of Lake Eyre has a very low gradient, with the exceptions of the channels (the Kalaweerina,

Warburton and Cooper grooves) formed where the largest rivers enter the Lake (Kotwicki 1986). When full, the lake is relatively shallow, with Lake Eyre North reaching a mean depth of 3.3 m, and Lake Eyre South a mean depth of 1.9 m, during the largest recorded flood event (Kotwicki 1986). Because of its flat base and shallowness, it is strongly affected by wind, to the point where it can become a 'roving' lake, with waters that advance and retreat, and therefore expose and cover large expanses of the lake bed (Torgersen 1984).

While the remoteness of Lake Eyre has resulted in few water quality datasets, some information exists regarding its salinity. As it fills, Lake Eyre becomes increasingly saline, with relatively fresh flows dissolving the salt crust (Habeck-Fardy & Nanson 2014). When completely full, crust dissolves entirely, resulting in a maximum surface salinity of 57 ‰ recorded towards the end of the 1974 fill, far exceeding that of sea water (35 ‰, Dulhunty 1977). Stratification may occur, with salinity of 50–300 ‰ recorded during 1974 in a ~1 m deep layer of water at the bottom of Lake Eyre (Ruello 1976). In the first month of filling during the 1984 flood, salinity levels rose to 25 ‰, but as evaporation dried the lake down, salinity rose rapidly to 272 ‰ (Williams & Kokkinn 1988). The temperature of the Lake's water is also expected to be high, with daytime surface temperatures of 20–25 °C, and night-time temperatures approximately 5 °C cooler (Bayly 1976; Barton & Takashima 1986). Other water quality variables have not been studied in detail, although a neutral pH (7.2) has been recorded (Ruello 1976).

Riverine Waterholes

Riverine waterholes, i.e. isolated, relatively deep segments of channel that water persists in post-flow (Fig. 8), represent the main 'permanent' aquatic habitat in the LEB, and are therefore vital for the maintenance of its aquatic biodiversity (Knighton & Nanson 1994; Sheldon *et al.* 2002; Hamilton *et al.* 2005; Davis *et al.* 2013; Costelloe & Russell 2014; McNeil *et al.* 2015). Here, permanent waterholes are defined as those channel segments that retain water for at least 12 months. While there are some LEB waterholes that retain water for much longer, and are not known to have dried since European pastoral settlement (Table 1; Fensham *et al.* 2011), most have dried at least once in the last 200 years (Wager & Unmack 2000). Most waterholes rely on flow events to replenish water and allow persistence. Exceptions to this rule occur rarely, but some riverine waterholes are additionally fed by springs, and even alluvial groundwater in some sand-bed rivers. Groundwater inflow

influences waterhole persistence in the Neales and Georgina, where groundwater enhances 17 of 22 waterholes, although not in the Diamantina or Cooper (Fensham *et al.* 2011; Costelloe & Russell 2014). Waterhole size varies greatly, with Cooper Creek waterholes found to have a mean length of 3.7 km (ranging from <50 m to >20 km) and mean width of 55 m (Knighton & Nanson 1994).

Figure 8. Eringa Waterhole, a typical riverine waterhole in the Macumba River in the Lake Eyre Basin, lined with *Eucalyptus* and with a clay base and turbid water.

The number of permanent waterholes varies among river systems, with the largest numbers in the Channel Country rivers, fewer in the shorter Desert and Western rivers and South-West creeks, and none in some tributaries, such as the Macumba (Table 1). Where present, waterholes are usually within the main river channel and are more common in, or even restricted to, upper reaches of rivers, due to two main factors. Topographical relief is often greater in upper reaches, which provides protection from evaporation, especially in places such as gorges (Fig. 9). For example, most permanent waterholes in the Finke River are in the upper–Finke, with few in the mid-Finke and likely none in the lower-Finke (Angus Duguid, *pers. comm.*). In other systems, such as the Channel Country rivers, waterholes are more common in upper and mid-reaches, where flow is greater, and are absent in lower reaches (Fensham *et al.* 2011). Waterholes also commonly occur at points of flow constriction or concentration, where different flows converge, and frequently occur in clusters (Knighton & Nanson 1994; Costelloe & Russell 2014).

The presence of waterholes is also dependent on the composition of the sediments of the channel bed, with sandy rivers too free-draining to retain surface water (Fensham *et al.* 2011). In some parts of the LEB, waterholes may sit upon rocky bedrock, which prevents leakage and maintains pools; such waterholes have been observed, for example, in the Neales and Finke Rivers (*pers. obs.*). However, in the Channel Country and much of the rest of the LEB, waterholes often lie on several metres of fine clay, which seals the basin (Hamilton *et al.* 2005). The presence of clay results in persistence high turbidity in many LEB waterholes (Fig. 8), even during extended drought periods (Bunn *et al.* 2006b). In contrast, waterholes in the western LEB are more often clear (Fig. 10), although turbid waterholes may be present in smaller systems (Davis *et al.* 2013). Following cessation of flow, the water quality in waterholes often shows a marked decline, with rising salinity and temperatures, and potential decreases in dissolved oxygen (Hamilton *et al.* 2005; Sheldon *et al.* 2010; McNeil *et al.* 2015).

Figure 9. A gorge-sheltered waterhole in Ormiston Gorge, in the upper Finke River of the Lake Eyre Basin.

Three interrelated factors govern waterhole permanence: frequency of inflow, depth, and rate of water loss (Fensham *et al.* 2011). Frequency of inflow differs among rivers depending on flow regime, but is also impacted by position in the river system and channel (Hamilton *et al.* 2005). Depth is highly variable among waterholes, but it has been predicted that a depth of ~4 m is required for permanence (Costelloe *et al.* 2007). Evaporation is the main cause of water loss, with annual loss modelled to be 1.5–2.5 m (Costelloe *et al.* 2007). Evaporation is lower when geographic features or riparian vegetation reduce exposure of the water's surface, and may also be reduced by increasing salinity (Hamilton *et al.* 2005; Costelloe *et al.* 2007; Fensham *et al.* 2011). As future climate change is expected to reduce the frequency of flow events in arid central Australia (Watterson *et al.* 2015), the permanence of these waterholes, and their vital role in supporting the persistence of aquatic biodiversity, may be threatened (Davis *et al.* 2013).

The Ecology of Lake Eyre Basin Waterbodies

Due to the extreme variability of rainfall in arid central Australia, the region's ecology is dominated by a 'boom and bust' cycle (Byrne *et al.* 2008; Van Etten 2009). Boom and bust refers to the 'boom' in productivity after a large rainfall event, followed by the 'bust' as the landscape dries and productivity crashes (Leigh *et al.* 2010; Sheldon *et al.* 2010). This cycle is especially important in river systems, but less so in springs that have continuous water inputs. In the LEB, rainfall events cause rivers to undergo massive changes, from dry channels with occasional waterholes to wide flowing waterways (Jenkins & Boulton 2003; Bunn *et al.* 2006b; Balcombe & Arthington 2009; Arthington & Balcombe 2011). The irregular and unpredictable cyclic shift, from dry to wet and back again, and associated environmental changes, determines which aquatic species can persist in the region, with only those with strong resistance and/or resilience traits able to maintain populations through time (Boulton & Suter 1986; Bogan *et al.* 2017).

While naturally unpredictable, the boom and bust cycle occurs more or less often in different parts of the LEB, depending on flood frequency. During long intra-annual droughts, bust periods can last many years, however when booms occur, they are often repeated the following year. This is because much of the LEB's rainfall, especially large, widespread rain that causes floods, occurs during La Niña phases of the El Niño Southern Oscillation phenomenon, which is usually protracted over multiple years (Kotwicki & Allan 1998;

McMahon *et al.* 2008a). Flood events in sequential years often result in the filling of Lake Eyre (Kotwicki 1986), but also provide a massive boost for aquatic biodiversity and productivity in rivers (Puckridge *et al.* 2000), with cumulative hydrological effects that ensure persistence of habitats and biodiversity in the LEB (Leigh *et al.* 2010).

The scale of flows is also unpredictable, and plays an important role in regulating diversity and ecological processes. While smaller in-channel flows are more frequent, and have important roles in sustaining habitat quality, it is the larger floods that overtop banks and spread to the floodplain that cause 'booms' (Leigh *et al.* 2010). While these floods connect the isolated waterholes along the channels, transport nutrients and sediments downstream, and facilitate dispersal of aquatic species, they also form vast lateral hydrological connections between the river and the floodplain, which benefits terrestrial and aquatic biota (Capon 2005; Kingsford 2006). While the ability to take advantage of these connections differs between species, it is also reliant on size of the flood, with larger floods having a longer duration and therefore providing more opportunities for productivity, growth, reproduction and dispersal (Leigh *et al.* 2010).

Figure 10. Two Mile Waterhole, a typical riverine waterhole in the Finke River in the Lake Eyre Basin. The benthic sediments are mainly gravels and sands and the water column is characterised by clear water and fringing and submerged aquatic vegetation.

Although the productivity boom caused by large floods occurs in both the terrestrial and aquatic environment, it is largely driven by aquatic production on the floodplain, which provides food for many terrestrial and aquatic consumers (Bunn *et al.* 2006b). Aquatic invertebrates move onto the floodplain to feed on algae and zooplankton, but food web analyses in Cooper Creek indicate that they do not consume significant quantities of terrestrial resources (Bunn *et al.* 2003). Fish also move into the floodplain to feed, with diverse and abundant resources resulting in shifts in diet from that consumed in disconnected waterholes (Thoms 2003). In Cooper Creek, most fishes feed on aquatic invertebrates while on the floodplain, but a few species feed on algae or terrestrial material (Balcombe *et al.* 2005). Fish also breed during booms, with many species showing very high juvenile recruitment after floods (Arthington & Balcombe 2011). The boom attracts waterbirds to the rivers of the LEB, often in the hundreds of thousands, which feed and may breed on the floodplains (Kingsford *et al.* 1999).

While Lake Eyre itself fills less frequently than any LEB rivers flow, the environmental changes are no less extreme. When full, Lake Eyre supports a range of flora and fauna, including emerged and submerged macrophytes, algae, zooplankton and fish in high abundance (Bayly 1976; McMahon *et al.* 2005). For example, during the fill event in 1974, it was estimated that the Lake supported over 40 million fish, mostly of two herbivorous species (Ruello 1976). The plentiful food also attracts thousands of waterbirds (Kingsford & Porter 1993). However, it is unclear if productivity within the Lake is high, or if most food resources are washed into the Lake from floods. Waterbirds tend to congregate around inflow locations into the Lake, suggesting that inflowing resources, rather than productivity, may be driving the system (Kingsford & Porter 1993). However, as these areas are also the least saline, due to relatively fresh inflow, the productivity may also be highest at these locations. Salinity in the Lake is too high for most invertebrates, and diversity of this group is low, being dominated by highly-tolerant chironomids and small crustaceans (copepods, ostracods, anostracans; Williams & Kokkinn 1988).

After a rainfall event, flows often cease within a few days or weeks, although larger rainfalls can cause flows to persist for months, and localised areas of the floodplains may be inundated for more than a year (Kotwicki 1986). In most parts of the channel, flow cessation is followed by a shift from an aquatic ecosystem to a terrestrial one, with fully aquatic species retreating

again to waterholes (Steward *et al.* 2012). Other taxa, including some aquatic invertebrates, have desiccation-resistant life-history stages that allow them to persist in the dry channel beds during periods of no flow (Strachan *et al.* 2015). However, most larger aquatic species, including all fish, do not have such abilities, and must survive within waterholes if they are to persist within the system (Wager & Unmack 2000). Within the LEB, and other arid regions in Australia and globally, waterholes function as refuges for aquatic biodiversity during periods of no flow (Hamilton *et al.* 2005; Sheldon *et al.* 2010; Davis *et al.* 2013). Refuges are here defined as sites to which biota retreat to, persist in, and may potentially expand from under changing environmental conditions (following Keppel *et al.* 2012).

As waterholes become disconnected and isolated from the floodplain and channel, the bust begins. Waterholes are often turbid, with limited light penetration, so productivity of aquatic plants within these environments is relatively low, with aquatic fauna relying on terrestrial inputs (Bunn *et al.* 2003). This includes inputs laterally transferred from the floodplain during inundation, as well as continual input from fringing vegetation. Most waterholes are lined by trees, usually *Eucalyptus camaldulensis* and *E. coolibah*, which provide large amounts of detritus (Hamilton *et al.* 2005). However, studies of food webs in Cooper Creek showed that terrestrial sources were largely insignificant contributors to the aquatic food web. Instead, large populations of molluscs, crustaceans, other invertebrates, and fish were supported by highly-productive filamentous algae that grow in the shallow littoral margins (Bunn *et al.* 2003). Waterhole morphology is therefore an important determinant of productivity; waterholes with more extensive littoral areas should have greater primary productivity, and support greater abundances and diversity of species (Sheldon *et al.* 2010).

The longevity of waterholes after flows cease is dependent on a range of factors, including size, as discussed above (Hamilton *et al.* 2005). As a waterhole dries down, conditions deteriorate and the number of species that can survive in it declines. This is largely due to the reduction in water volume, which concentrates solutes (such as salt) and also decreases the buffer against more rapid changes in temperature, dissolved oxygen and other abiotic variables (Hamilton *et al.* 2005). In Lake Eyre, massive fish kills have been observed during the drying down phase, which are suggested to be due to a rise in salinity, decrease in dissolved oxygen, extreme temperatures or rapid fluctuations in temperature (Ruello 1976). In individual waterholes, water temperature during the day are typically 12–19 °C,

increasing to 25–30 °C during the summer months (Glover 1982). However, extremes are more likely in smaller waterholes – in winter shallow areas can drop below 10 °C overnight, while in summer they may exceed 40 °C during the day (Glover 1982). Biotic interactions, such as competition and predation, can also limit which species can persist in a waterhole (Sheldon *et al.* 2010). For example, poorer competitors may be excluded from one large waterhole, but can persist in a smaller waterhole with poorer water conditions if they exhibit greater tolerance of environmental extremes (Bogan *et al.* 2017).

Indeed, Sheldon *et al.* (2010) suggest that no one type of refuge (waterhole or other waterbody) can provide suitable abiotic and biotic conditions for all aquatic biodiversity. Instead, a spatial and temporal mosaic of waterholes, each providing different abiotic and biotic conditions, would be required for the persistence of all species (Bunn *et al.* 2006a). Robson *et al*. (2008) identified different types of refuges, including 'ark' refuges that "contain an assemblage of species that is representative of aquatic biodiversity at the landscape scale" and 'polo club' refuges that support only the subset of taxa with traits required to persist in the refuge's environment. This concept was explored in the Neales River, where refuge types were identified based on fish assemblages (McNeil & Schmarr 2009; McNeil *et al.* 2011b). These included one 'ark' refuge, which contained all native fishes, and several 'polo clubs' with high salinity where only tolerant fishes persisted. In addition, temporary 'disco' refuges that provided habitat for fishes during seasonal dry periods, but dried up during longer droughts, were identified (McNeil *et al.* 2011b).

The role of refuges in supporting the persistence of arid zone aquatic biodiversity were further explored by Davis *et al*. (2013), who identified evolutionary refugia and ecological refuges across the Australian arid zone. Evolutionary refugia are those habitats that are permanent on a millennial timescale, such as groundwater-fed springs, and often support vicariant relicts and short-range endemics. The climate of these refugia is often decoupled from that of the surrounding landscape, resulting in a stable microclimate (Davis *et al.* 2013). Ecological refuges, which include riverine waterholes, are unlikely to be permanent and instead exist on shorter time-scales, typically days to decades. Ecological refuges also vary through space, and while they contain a range of aquatic and terrestrial taxa, they rarely support short-range endemics (Fensham *et al.* 2011). Ecological refuges are strongly linked to the regional climate and governed by boom and bust dynamics (Davis *et al.* 2013).

In the LEB, most fishes utilise refuges to support metapopulation dynamics, with the presence of a patchwork of refuges through space and time facilitating persistence (Unmack 2001b; Sheldon *et al.* 2010; Davis *et al.* 2013). Because these refuges are vital for the persistence of most aquatic biodiversity in the LEB, threats to refuges may affect many species (Brim Box *et al.* 2008). Refuges are likely to persist through future climatic changes if local climate decoupling maintains acceptable environmental conditions. As most evolutionary refugia are groundwater-fed, they are less likely to be affected by a drying climate (one possible future scenario; Watterson *et al.* 2015) than the more precipitation-reliant ecological refuges (Davis *et al.* 2013). Pleistocene aridification of the LEB has resulted in a situation where groundwater systems are no longer in equilibrium, and are in a state of net discharge (Hatton 2001). As this state is unsustainable, the long-term future of these refugia may be in doubt. Many other anthropogenic impacts (see following section) are also likely to affect the future viability of aquatic refuges in the LEB.

Anthropogenic Influences on Aquatic Ecosystems in the Lake Eyre Basin

The LEB has a long history of human presence, with Aboriginal people inhabiting the region for at least the last 30,000 years (Thorley 1998). However, occupation of the more arid areas likely occurred more recently, during the Holocene. During this time, and up until European colonisation, a number of Aboriginal groups lived in the LEB. Most were subsequently displaced by pastoral settlement in the second half of the 19th Century (Watson 1998).

Today, the LEB has a human population of around 60,000, of which around half live in Alice Springs (Lake Eyre Basin Ministerial Forum 2011). Settlements are largely restricted to the margins of the LEB (Alice Springs, Coober Pedy), or along the larger Channel Country rivers (Winton, Longreach, Windorah, Innamincka; Fig. 11), with a sparse population elsewhere. The major land use in the LEB is stock grazing, with cattle and sheep grazing over 80% of the Basin. Other land-uses include mining, oil and gas, and tourism, with some small conservation reserves also present (Fensham *et al.* 2011). In terms of land tenure, approximately 71% of the LEB is pastoral leasehold, 12% private freehold, 10% parks and reserves, 5% crown land, and 2% Aboriginal land (Lake Eyre Basin Ministerial Forum 2011). Native title over Lake Eyre and immediate surrounds is held by the Arabana people, but a number of other groups hold title over other parts of the LEB (Sutton 1995).

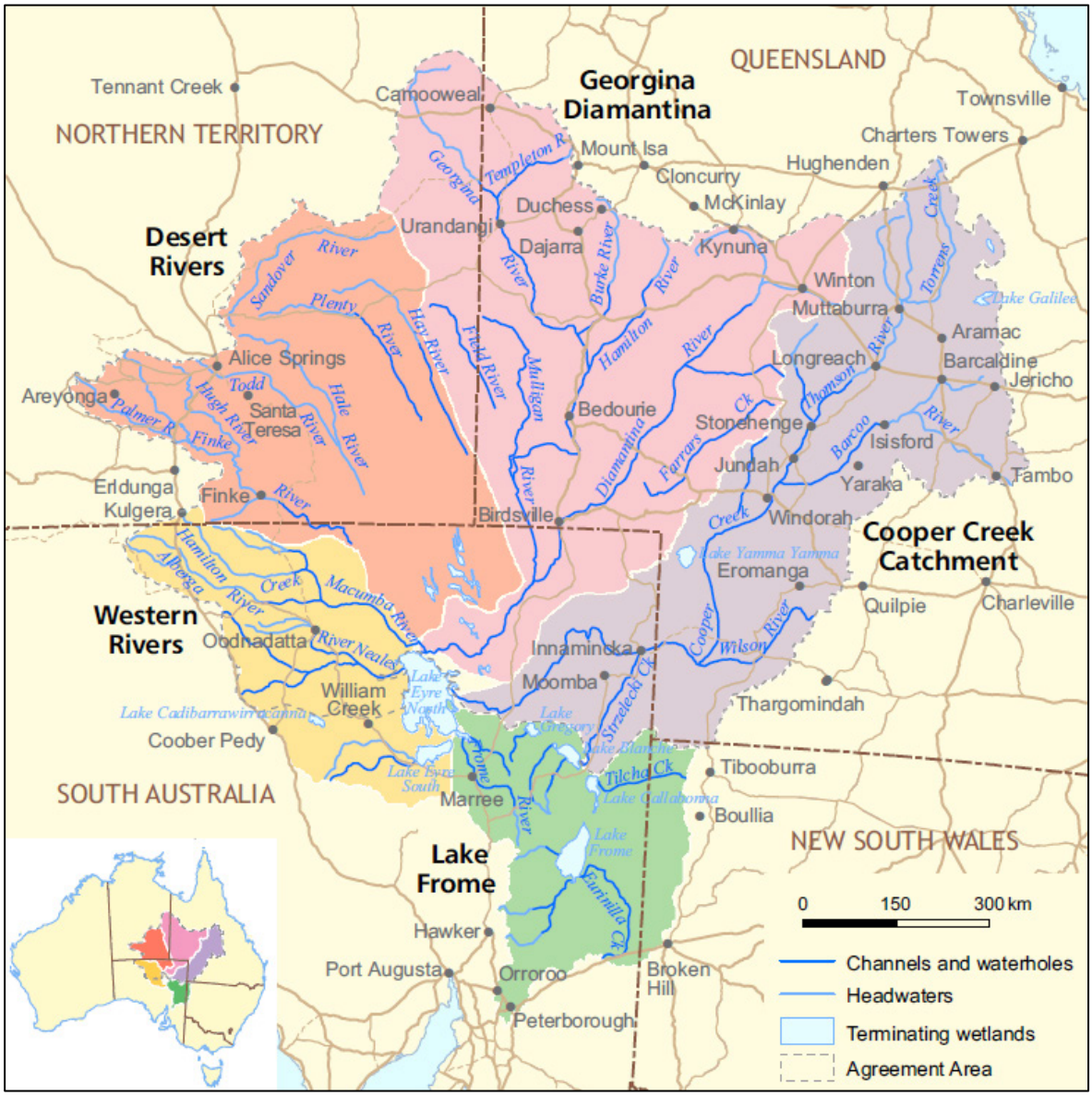


Figure 11. Map of the Lake Eyre Basin, showing its division into five major drainages for management purposes (from Lake Eyre Basin Scientific Advisory Panel 2008). The Lake Eyre Basin Intergovernmental Agreement applies to the area within the dashed line, which excludes the southern portion of the LEB.

The LEB's waterbodies are governed by the Lake Eyre Basin Intergovernmental Agreement, to ensure sustainable management of the LEB's water and related natural resources, and avoid downstream impacts on the environments and human activities (Lake Eyre Basin Ministerial Forum 2011). It aims to promote ecologically sustainable development, and recognises the ecological and cultural significance of the region. The agreement was signed between the Australian Commonwealth government and the state governments of Queensland and South Australia in 2000, and joined by the Northern Territory government in 2004 (Lake Eyre Basin Ministerial Forum 2011). While most of the LEB is covered by

this agreement, the southern portion is excluded, including Lake Eyre itself (Fig. 11). While this collaborative approach has many strengths, when one party makes unilateral decisions outside the Agreement weaknesses are clear, as demonstrated when Queensland moved to open development of LEB rivers following a change in government, as discussed by Kingsford *et al*. (2014).

The LEB is the largest unregulated arid region river basin globally, with no river damming or large-scale water extraction (Puckridge *et al.* 1998; Hamilton *et al.* 2005; Costelloe & Russell 2014). Recent changes to water extraction legislation may change this however, with withdrawal of water potentially affecting downstream waterhole persistence and other flood processes, especially during small to medium flow events (Sheldon *et al.* 2010; Arthington & Balcombe 2011). Overall, the hydrology and waterbodies of the LEB have experienced minimal impacts from anthropogenic activity compared with other basins in Australia (Kingsford *et al.* 2014). The most notable impacts stem from barriers to flow, groundwater extraction, and degradation of aquatic habitats, although all occur largely at the local scale, with no widespread degradation.

Barriers to flow occur in a number of places throughout the LEB, and can have large impacts, especially on smaller flow events that are needed to replenish isolated waterholes (Hamilton *et al.* 2005; Sheldon *et al.* 2010). These low-flow events can be disrupted by small impoundments, raised roads or railway tracks, and other interferences with channel structure (Hamilton *et al.* 2005). Barriers may also create new aquatic habitats, such as where water is trapped and pools (Fig. 12, *pers*. *obs*.). Barriers also impede the movement of aquatic animals, including fish, however this is unlikely to pose a long-term problem as larger flow events will overtop such barriers and hydrological connectivity will be (temporarily) restored.

Groundwater extraction, via local bores, has been extensively utilised throughout the LEB to support pastoral and other activities (James *et al.* 1999). Beginning in the 1870s, widespread drilling resulted in at least 23,000 bores across arid Australia, with a large number of these in the LEB (James *et al.* 1999). In many cases, these were free-flowing bores, where groundwater flowed freely to the surface, forming large pools and even wetlands, and creating new aquatic habitat (Wager & Unmack 2000; Fensham *et al.* 2011). Unfortunately, over-exploitation led to many of these bores running dry. Spring discharges have also declined as a result of bore development in, and some LEB springs have ceased to

flow (Habeck-Fardy & Nanson 2014). To reduce this issue, most bores are now capped (or are planned to be capped), with valves regulating flow (Department of Agriculture and Water Resources 2017).

Figure 12. A pool formed as a result of a raised roadway in the Lake Eyre Basin, in which several fish species were found. While a culvert is present, it was disconnected from the main pool.

The introduction of cattle and other exotic mammals into the LEB has had major impacts on the region's waterbodies. The LEB holds between 500,000 and 1,000,000 head of cattle, as well as a large number of sheep, depending on temporally-variable, rain-driven productivity (Lake Eyre Basin Ministerial Forum 2011). In addition, large numbers of feral hoofstock are also present, including pigs, goats, donkeys, horses and dromedary camels (Brim Box *et al.* 2008). In 2008, the camel population was estimated to be more than 1,000,000 across arid Australia, although subsequent control efforts reduced this number (Saalfeld & Edwards 2010; Brim Box *et al.* 2016). Cattle, camels, and other hoofstock have a major impact on LEB waterholes, especially during extended drought periods, including trampling, pugging, fouling, muddying, destabilising, drinking, grazing and browsing (Wager & Unmack 2000; Fig. 13; Brim Box *et al.* 2016). These impacts can lead to poorer water quality,

eutrophication and loss of habitat for aquatic species, and loss of water sources for other animals and riparian vegetation (Brim Box *et al.* 2010; McBurnie *et al.* 2015). In addition, there is evidence that camels can drink waterholes entirely dry, a major threat to the persistence of aquatic biodiversity (Brim Box *et al.* 2008).

Figure 13. Cattle damage, pugging and loss of riparian vegetation is reduced inside (left) relative to outside (right) a recently erected exclusion zone around a spring on a cattle station in the Lake Eyre Basin.

Other exotic species have had less apparent effects on the LEB's waterbodies, the European rabbit being the other notable impact (Brim Box *et al.* 2008). This species did not drink the waterholes dry, but fed intensively on riparian vegetation, causing increased evaporation of waterholes. Fortunately, the introduction of rabbit calicivirus in the mid-1990s has resulted in population decline of over 80% for this species in central Australia (Edwards *et al.* 2002). The most common native large mammals are several species of kangaroos, which are not thought to impact the LEB's waterbodies significantly, despite population increases since European settlement (Wager & Unmack 2000).

The impacts of anthropogenic climate change are already being felt in the LEB, with mean surface air temperatures increasing by 0.9–1.0 °C from 1910 to 2013 (Watterson *et al.* 2015). Future climate change scenarios have been modelled across the Basin, which predict higher temperatures, more warm days, reduced humidity and increased evaporation rates (Watterson *et al.* 2015). Rainfall is expected to decrease in the south of the LEB, but may intensify in the north as tropical cyclones become more severe. The amount of time spent in drought conditions is expected to increase in the most extreme model scenarios (Watterson *et al.* 2015). Overall, these changes are likely to have a greater impact on the waterbodies in the south of the LEB, but all river systems will likely experience reduced hydrological connectivity, longer periods of no flow and fewer refugial waterholes that persist between flow events. Similar impacts are expected in desert freshwaters globally (Chiu *et al.* 2017).

Conclusion

The Lake Eyre Basin is an extreme arid environment, and its rivers represent one of the most dynamic and challenging habitats for freshwater biota on the planet. Unlike the waters of many of the world's arid regions, the LEB's rivers do not arise in more mesic areas, and nor are they fed by predictable seasonal inputs (Kingsford 2006). Instead, its rivers are fed almost entirely by localised and variable rainfall events, which drive the dynamic 'boom and bust' ecology of the LEB. On a global scale, the LEB represents one of the last examples of an unregulated, variable dryland river system, and certainly the largest.

An understanding of the ways in which multiple processes interact in the LEB, including climate, hydrology and ecology, is essential for understanding how freshwater biota persist in this extreme environment. Many of these processes differ across the LEB. Climatic gradients exist, for example the variability in rainfall is greatest in the north, with winter rains becoming more common in the far south. Hydrology also differs, partially as a consequence of climate. However, some aspects of hydrology are not climatically-driven, for example, the number and size of waterholes, which largely results from geomorphology. The ways in which ecological processes differ are less clear, but aquatic communities differ among river systems (Fensham *et al.* 2011), suggesting that ecological dynamics will not be identical. Studies that utilise the 'natural experiments' provided by the LEB can explore how these processes contribute to the enduring persistence of aquatic life in this arid environment.

Understanding how species persist, and how they may persist in the future, is of great importance in a changing world. Future climate change will increase the variability of rain events and the length and frequency of droughts, making persistence more difficult for aquatic taxa in the LEB (James *et al.* 2017). Other anthropogenic impacts are also expected to reduce habitat availability and structural connectivity, resulting in one of the world's most extreme environments becoming even more challenging for freshwater biodiversity. Thus, studies of the LEB should not only include exploration of how multiple processes contribute to species' persistence, but also investigations into the potential impacts of changes on future persistence. The understandings arising from such research can inform conservation management, both in the LEB and in arid regions globally.

Chapter Four

The Desert Life Aquatic:
The Ecology of the Fishes of the Lake Eyre Basin

Introduction

Despite deserts being among the most inhospitable environments on Earth, almost all support aquatic habitats and a surprising diversity of aquatic life. Fish are particularly abundant, with over 1,100 species known to inhabit arid regions (Kingsford 2006). Spatial and temporal variability of water drives the ecology of fishes in arid regions, and requires traits that allow persistence through droughts and floods (Minckley & Deacon 1991; Wager & Unmack 2000; Kingsford 2006; Humphries & Walker 2013; Murphy *et al.* 2015). Desert fishes do not differ significantly to fishes in more mesic regions, and generally lack unique adaptations (Deacon & Minckley 1974). In fact, most desert fishes are generalists with wide environmental tolerances (Kingsford 2006). Most are highly reliant on dispersal, utilising networks of habitat to persist (Unmack 2001b). Together, these traits allow survival in some of the most extreme environments on the planet.

Australia's Lake Eyre Basin (LEB) is one of the world's driest and most dynamic arid regions (Chapter Three). It is, therefore, an extreme environment for fish. However, it is home to a variety of fishes, which can be seen as unique or representative of global arid zone fish faunas, depending on the characteristics considered. These fish represent a range of different biologies and ecologies, and exhibit life-history strategies common to all arid zone fish faunas. However, the origins and evolutionary histories of Australia's freshwater fishes are distinctive, and have shaped a fauna that differs from those in other arid regions.

This chapter reviews the fishes of the LEB, focussing on their evolutionary origins and biogeographic histories, biology and ecology, and the strategies they may utilise to persist. There is particular focus on the taxa that are the subject of further study in this thesis. The effects of anthropogenic influences are also considered, to identify threats to the continued persistence of the distinctive fish fauna of the LEB.

The Fish Fauna of the Lake Eyre Basin

In contrast to the striking diversity observed in many other groups of Australian fauna, the continent's freshwater fish fauna is relatively depauperate. While total species numbers are frequently revised (usually upwards), the current richness of Australian freshwater fishes stands at ~300 (Humphries & Walker 2013). For comparison, the contiguous states of the USA cover a slightly smaller land area than Australia, but contain almost 1,000 fishes (Froese & Pauly 2017). The number of species inhabiting arid regions is also low: Australia has 46 recorded desert fishes, compared with 269 in North America (Kingsford 2006). The low richness in Australia may be partially due to cryptic species and a lack of research into many groups, which may mask the true diversity (Faulks *et al.* 2014). While only two of the 21 freshwater fish families present in Australia are endemic, endemicity rises at lower taxonomic levels: 32 of 89 genera are endemic, as are ~74% of species (Unmack 2013).

In Australia, arid inland regions tend to be less speciose in fishes than those in more mesic or tropical locations. However, they do harbour a greater proportion of endemics, due to the longer isolation of inland basins compared with coastal basins (Unmack 2001a). The LEB contains thirty-three native fishes, of which seventeen are endemic (Wager & Unmack 2000; Unmack & Dowling 2010). The species represent eleven diverse families, each typically represented by one or two genera, some with very shallow species radiations (Table 1). The terapontids are the main exception, with four genera present, each with a single species. However, the taxonomy of this group is not well resolved, and some of these genera may be paraphyletic (Peter Unmack, *pers. comm.*).

Of the fishes that are native (but not endemic) to the LEB, most have a very wide distribution across northern Australia through a variety of biomes (Unmack 2013). In contrast, LEB endemic species are usually range-restricted, with eight found only in localised spring systems. Across the LEB, there is a notable lack of congeneric sympatry (Unmack 2013). This is largely driven by divergences occurring across geographic barriers or between springs with extreme environmental conditions (e.g. Unmack & Dowling 2010). Further, individual rivers generally have unique fish assemblages, despite being hydrologically connected and having similar environments (Glover 1982). For example, the adjacent Cooper and Georgina-Diamantina systems have distinct faunas – with eight fish species not shared between the systems (Wager & Unmack 2000). These distributional patterns are less

common in Australia's tropical and mesic basins, and may reflect either historical colonisation of just one part of the LEB, or more recent extinction and recolonisation patterns.

Table 1. Orders, families, species and common names of fish native to the Lake Eyre Basin. Asterisks indicates species endemic to the LEB; species in bold are focal taxa of this study.

Order	Family	Scientific Name	Common Name
Siluriformes	Plotosidae	*Neosiluroides cooperensis*	Cooper Creek Catfish*
		Neosilurus gloveri	Dalhousie Catfish*
		Neosilurus hyrtlii	Hyrtl's Catfish
		Porochilus argenteus	Silver Tandan
Clupeiformes	Clupeidae	*Nematalosa erebi*	**Bony Herring**
Osmeriformes	Retropinnidae	*Retropinna semoni*	Australian Smelt
Perciformes	Ambassidae	*Ambassis* sp.	North-West Glassfish
	Percicthyidae	*Macquaria* sp.	Lake Eyre Golden Perch*
	Terapontidae	*Amniataba percoides*	**Barred Grunter**
		Bidyanus welchi	Welch's Grunter
		Leiopotherapon unicolor	**Spangled Perch**
		Scortum barcoo	Barcoo Grunter
	Eleotridae	*Hypseleotris klunzingeri*	Western Carp Gudgeon
		Hypseleotris sp.	Midgley's Carp Gudgeon
		Hypseleotris sp.	Lake's Carp Gudgeon
		Mogurnda clivicola	Barcoo Mogurnda*
		Mogurnda larapintae	Finke Mogurnda*
		Mogurnda thermophila	Dalhousie Mogurnda*
Atheriniformes	Atherinidae	*Craterocephalus centralis*	**Finke Hardyhead***
		Craterocephalus dalhousiensis	Dalhousie Hardyhead*
		Craterocephalus eyresii	**Lake Eyre Hardyhead***
		Craterocephalus gloveri	Glover's Hardyhead*
		Craterocephalus stercusmuscarum	Fly-specked Hardyhead
	Melanotaeniidae	*Melanotaenia splendida tatei*	Desert Rainbowfish*
	Pseudomugilidae	*Scaturiginichthys vermeilipinnis*	Red-finned Blue-eye*
Gobiiformes	Gobiidae	*Chlamydogobius eremius*	**Desert Goby***
		Chlamydogobius gloveri	Dalhousie Springs Goby*
		Chlamydogobius japalpa	**Finke Goby***
		Chlamydogobius micropterus	Elizabeth Springs Goby*
		Chlamydogobius squamigenus	Edgbaston Spring Goby*
		Glossogobius aureus	Golden Goby

The Origins of the Lake Eyre Basin Fishes

The origins of the LEB's, and indeed Australia's, fish fauna are not well understood, although the evolutionary distinctiveness of the continent's freshwater fishes has long been recognised. Globally, the vast majority of freshwater fishes are of the superorder Ostariophysi. This group dominates the freshwater fish faunas of Africa, Europe, Asia and the Americas, including their arid regions (Berra 2001). The other major superorder of fishes is the predominantly marine Acanthopterygii, which make up most of the Australian freshwater fish fauna. In fact, many Australian fishes are the only freshwater representatives of families that are otherwise marine fishes of the Indo-Pacific region (Pusey *et al.* 2004). This unique situation has arisen from the lack of freshwater connections, required by most ostariophysians for dispersal, between Australia and other continents (Unmack 2013). This has meant that Australia's freshwater habitats have been largely colonised by marine acanthopterygians. The only exceptions are two families of largely-marine ostariophysian catfish, and the saratoga and lungfish, which are Gondwanan relicts with a vicariant origin potentially dating to 150 Mya (Allen *et al.* 2002). Of the exceptions, only members of the catfish family Plotosidae are present in the LEB, with all other fishes being acanthopterygian.

It is unclear when the marine ancestors of Australia's freshwater fishes arrived, due partially to the paucity of fossils, although some groups are thought to have colonised millions of years ago (Unmack 2001a; Allen *et al.* 2002; Pusey *et al.* 2004). Indeed, it has been suggested that the majority of today's genera were present for most of the last 65 million years (Hills 1958). The few fossils studied to date indicate a variety of freshwater fishes were present 45 Mya, including members of Percichthyidae (Hills 1943; Turner 1982; Unmack 2013; Faulks *et al.* 2014). Fossils of larger fish (>20 cm) are more likely to be preserved (Unmack 2001a), but a few smaller fossils have been identified, including of Terapontidae dated to the Eocene (Turner 1982). A variety of catfish fossils have been dated to the Miocene (Pledge 1984). Molecular studies also provide evidence of evolutionary origins. A study of Australia's most speciose fish genus, *Craterocephalus* (Atherinidae), dated the initial freshwater diversification of this group to 49–75 Mya (Unmack & Dowling 2010).

Following colonisation, many families have experienced evolutionary radiations, perhaps resulting from environmental and climate changes in Australia (Pusey *et al.* 2004). While clear evolutionary history has not been elucidated for most Australian fishes, it may be

further complicated by the repeated marine transgressions, and other disturbances, which would likely have caused extinctions and allowed additional colonisations by estuarine species to occur (Pusey *et al.* 2004). Such disturbances may explain the relatively depauperate fauna, although this may also result from the general aridity of Australia, which restricts the niche space required for diversification (Faulks *et al.* 2014).

How and when fishes arrived in the LEB is largely unknown. Fossil evidence suggests that the catfish of the family Ariidae were present in the Miocene (Pledge 1984), but this family is no longer found in the LEB. The speciose goby genus *Chlamydogobius* may have colonised during the Miocene marine transgressions (Miller 1987), but this is largely speculative. Some evolutionarily distinct LEB fishes have few close relatives and may have existed in the LEB for a very long period of time. For example, the endemic Cooper Creek catfish, the only member of its distinctive genus, is likely an ancient resident (Unmack 2013). The Lake Eyre and Finke hardyheads appear to have diverged from their nearest relative, more than a thousand kilometres away in the Pilbara region of Western Australia, around 19 Mya, and aridity has likely isolated them in the LEB since (Unmack & Dowling 2010).

In contrast to these ancient endemics, many other LEB fishes appear to have arrived from adjacent basins (see Chapter Three Fig. 1) relatively recently, with most divergences between LEB fishes and their relatives dated to the Pleistocene, i.e. in the last 2.6 My (e.g. Hughes *et al.* 2009). Connections appear to have existed to most surrounding Basins, especially to the north and east (Unmack 2013). These connections likely occurred through areas of low divide, albeit of unknown location (Thacker *et al.* 2007). The many aquatic species shared between the LEB and the Murray-Darling Basin (MDB) to the east indicate multiple recent connections (Hughes *et al.* 2009). These connections span a range of dates – Australian smelt populations in the two basins diverged ~1.6 Mya, bony herring split ~120 kya, Hyrtl's catfish ~70 kya and golden perch ~58 kya (Huey *et al.* 2006; Hughes & Hillyer 2006; Hughes *et al.* 2009; Faulks *et al.* 2010a). Other fishes also diverged from MDB relatives, including rainbowfish and western carp gudgeon (Thacker *et al.* 2007). When also considering other aquatic taxa (e.g. crustaceans and molluscs), there is a clear pattern of more dispersive species diverging more recently, indicating multiple connections in the past, although not utilised by all species (Hughes *et al.* 2009).

Connections to the north and north-east also occurred, with a number of fishes showing recent divergences from relatives in tropical rivers (Unmack 2013). To the north, links from rivers in the Gulf of Carpentaria Basin (GCB) to the north-eastern LEB appear to have facilitated dispersal of north-west glassfish (Huey *et al.* 2011a). These events occurred at different times, and resulted in distinct lineages establishing in the LEB: one in the Georgina River and one in the Diamantina River and Cooper Creek. A similar pattern was observed for bony herring, with colonisation events occurring ~350 kya and 162 ~kya from the GCB to LEB, resulting in two divergent LEB clades (Masci *et al.* 2008). Several other species, including rainbowfish (Unmack 2005) and the mogurndas (Adams *et al.* 2013) also appear to have had connections to the north.

Finally, there is also evidence of connections to the north-east, with several studies noting exchanges of fishes between the LEB and the Burdekin Basin on the east coast (Unmack 2013). The LEB population of Midgley's carp gudgeon are most closely related to those in the Burdekin, but are also related to the MDB population, and the colonisation history of this species is unclear (Thacker *et al.* 2007). Similarly, the LEB population of fly-specked hardyhead appears to have recently diverged from the Burdekin population (Unmack & Dowling 2010). Within the LEB, these two species are restricted to Cooper Creek, suggesting that following colonisation neither was able to disperse throughout the wider Basin. Other species appear to have utilised connections between the Burdekin and LEB too, including the rainbowfish and north-west glassfish (Unmack 2013).

While studies have found LEB fishes to be either relatively distinct or recently diverged, evolutionary histories are poorly known for almost all species (but see Faulks *et al.* 2010a). The colonisation history of the LEB is complicated by multiple colonisations (and extinctions) of fishes, with several well-studied species showing evidence of colonisation from multiple drainages (e.g. bony herring). Extinctions in adjacent drainages, notably in the arid Western Plateau drainage, make tracing histories even more difficult. Regardless of past connections, there is strong evidence that there are no contemporary hydrological connections between the LEB and other basins (Kotwicki 1986; McMahon *et al.* 2005). In addition, no studies have found contemporary connectivity between fish populations in the LEB and elsewhere, even among highly-dispersive species (Unmack 2013). So, it is likely that LEB fishes are isolated from populations in other basins, and must persist *in situ*.

The Biology of the Lake Eyre Basin Fishes

Desert fishes possess a range of traits to persist in arid environments. Most are relatively small, short-lived and opportunistic (Kingsford 2006). All have ecologies driven by the spatial and temporal variability of water, and exhibit adaptations that allow them to persist through droughts and take advantage of floods (Minckley & Deacon 1991; Humphries & Walker 2013). These 'boom and bust' periods (see Chapter Three) also dictate the life-history events of many species, including migration and spawning (Puckridge *et al.* 2000). Many desert fishes have broad environmental tolerances, which are most important during prolonged droughts when water conditions deteriorate (Deacon & Minckley 1974; Glover 1982). Many also have strong dispersal capabilities, used during floods (Unmack 2001b). Overall, these adaptations allow desert fish to persist in one of the most extreme environments on Earth.

The fishes of the LEB are ecologically similar to other arid zone fishes, and relatively distinct from the wider Australian fish fauna. For example, LEB fishes are generally smaller-bodied in comparison with those in more mesic parts of Australia, but are broadly comparable in size with the North American desert fish fauna (Blanchet *et al.* 2010a). While many Australian fishes are very long-lived, few LEB fishes are, with most having generation times of one to two years (Pusey *et al.* 2004). LEB fishes can be divided into widespread generalists and desert specialists, although many of the latter are restricted to springs (Wager & Unmack 2000) and are not considered in detail here. This division is seen in fish communities of desert freshwaters globally, with widespread generalists typically more numerous than specialists (Kerezsy *et al.* 2017).

LEB fishes tend to have wide diets, with most omnivorous and few strictly herbivorous (Wager & Unmack 2000; Pusey *et al.* 2004). During drought periods, when river fishes are restricted to waterholes, low-value food items, such as detritus and small zooplankton make up the majority of their diets (Balcombe *et al.* 2005). Diets change during boom periods, when many fish move onto floodplains and feed on higher-value food generated by greater aquatic productivity (Bunn *et al.* 2003; Thoms 2003). For example, bony herring fed primarily on detritus during low-flow periods, but on the floodplain switch to an algal diet (Balcombe *et al.* 2005). Few species utilise terrestrial inputs as a primary food source, but some feed on terrestrial invertebrates during boom periods (Balcombe *et al.* 2005). Several

fishes, including spangled perch and barred grunter, incorporate other fish into their diet, although most LEB fishes are gape-limited and their small size largely restricts their fish diet to larvae and juveniles (Pusey *et al.* 2004; Balcombe *et al.* 2005). The presence of piscivorous fishes may exclude some smaller fishes from aquatic habitats, although this has not been clearly demonstrated in the LEB.

As well as influencing diet, floods also trigger reproductive behaviours in many LEB fishes, with spawning commonly occurring soon after flows resume (Balcombe *et al.* 2007). This allows larvae and juveniles to utilise the floodplain as a nursery and results in abundances of many fishes rising rapidly (King *et al.* 2003; Arthington & Balcombe 2011). Because many LEB fishes mature within their first year (Pusey *et al.* 2004), sequential flood years can result in very high abundances of many species (Leigh *et al.* 2010). During drought, when productivity declines and water conditions deteriorate, population sizes are strongly reduced (Balcombe & Arthington 2009). The boom and bust ecology of the LEB is therefore strongly reflected in the demographics of fishes, and similar to temporal fluctuations seen in many desert fish populations worldwide (e.g. Medeiros & Maltchik 2001). Most LEB fishes can also reproduce during periods of no flow, although recruitment is lower than in booms (Kerezsy *et al.* 2011).

As in other arid regions, LEB fishes invest more in quantity of offspring than quality (Kingsford 2006). The fish assemblage of the LEB's Cooper Creek demonstrated far less parental investment in offspring than a comparable assemblage in a river in the Australian tropics (Puckridge 1999). Many species produce extremely large quantities of eggs; this includes the bony herring, which lays up to 880,000 eggs per spawning (Puckridge & Walker 1990). Many larger species spawn in congregations, with eggs released into the water, while smaller fishes lay small groups of eggs onto vegetation or other surfaces (Wager & Unmack 2000; Pusey *et al.* 2004). Some smaller species, including gobies and gudgeons, guard eggs until they hatch (Wager & Unmack 2000). LEB fishes do not practice parental care of hatchlings (Pusey *et al.* 2004; Humphries & Walker 2013).

Previous studies of freshwater fishes have categorised species into reproductive strategies, based on fundamental demographic trade-offs (Winemiller & Rose 1992). Three strategies were defined by Winemiller (1992): opportunistic, periodic, and equilibrium. Opportunistic

species are generally small, rapidly-maturing and short-lived, and produce large numbers of offspring when conditions are favourable in a stochastic environment. Periodic species are larger, longer-lived, and highly fecund – capable of producing massive amounts of offspring when conditions are favourable in more predictable environments. Equilibrium species tend to be of intermediate size, and invest more heavily in offspring quality, including production of fewer, larger offspring and parental care, typically in relatively more stable environments. The reproductive strategies of a subset of LEB fishes have been hypothesised by McNeil *et al.* (2011b; see Table 2). Larger fishes were suggested to be opportunistic/periodic species, smaller fishes opportunistic, and just one species, the desert goby, an equilibrium species. These categorisations take into account both reproductive traits of the species, and the habitats they tend to inhabit within the LEB.

While floods offer a boom period for fishes, conditions rapidly deteriorate during droughts, with fish restricted to riverine waterholes and springs as rivers cease to flow (Sheldon *et al.* 2010). In extended drought, many smaller waterholes dry out completely, and only the largest waterbodies persist through to the next flood (Knighton & Nanson 2000). Accordingly, fish persistence in deserts is often thought to be facilitated by aestivation – an ability to enter a state of torpor or dormancy for extended periods of time (Richards 2010). While one group of Australian fishes (Galaxiidae) can aestivate, they have never been recorded in the LEB and there is no observational or experimental evidence for any LEB fishes (or their eggs) being able to aestivate (Wager & Unmack 2000). In fact, with the singular exception of the African lungfish, there is no evidence of any desert fishes worldwide being able to aestivate (Kingsford 2006). Successful aestivation requires a number of adaptations, including the ability to reduce metabolic rate, store products of protein catabolism, control water loss and exchange respiratory gases in air (Pusey *et al.* 4). Although the physiology of few LEB fishes has been investigated, it is unlikely that any possess all these traits (Pusey *et al.* 2004).

Without aestivation, the persistence of LEB fishes relies on their ability to survive in waterholes, despite the presence of these habitats varying over space and time (Sheldon *et al.* 2010). Two aspects of fishes' biology is thought to be of primary importance to their long-term persistence: environmental tolerances and dispersal abilities, discussed below.

Environmental Tolerances

During flow periods, the environmental conditions of the LEB waterbodies are relatively benign, and pose little challenge to fishes. However, after flows cease and waterholes become isolated, water conditions deteriorate, and continue to do so until flows resume (see Chapter Three). During droughts, environmental tolerance becomes increasingly important, as changes in different aspects of water quality differentially impact fishes, depending on their physiological tolerances (Glover 1982). For example, following cessation of flows in Cooper Creek, the most common fishes (catfishes and Barcoo grunter) declined in abundance, while the more tolerant bony herring became the most common species (Balcombe & Arthington 2009). These assemblage changes are common in LEB waterholes, and are often driven by aspects of water chemistry, the most important to fishes including increased temperature and salinity, and decreased oxygen levels (Hamilton *et al.* 2005).

Many LEB waterholes are relatively exposed and shallow, so water temperatures are susceptible to changes in air temperature (Glover 1982; McNeil *et al.* 2015). During winter, water temperatures are typically 12–19 °C, and may drop as low as 5 °C overnight. In summer, temperatures may reach over 40 °C, with a usual daytime range of 25–30 °C (Glover 1982). Thus, fish must be able to survive a wide range of water temperatures. The desert goby, an arid zone specialist, can tolerate a temperature range of at least 5–41 °C (Glover 1971). In contrast, some widespread species have narrower tolerances, and may be restricted to larger waterholes where temperatures are less extreme. For example, the north-west glassfish can only tolerate temperatures of 11–33 °C (Glover 1982).

Salinity levels vary greatly across LEB waterbodies. While larger waterholes are typically relatively fresh (salinity <1 ‰), smaller ephemeral pools frequently have salinities greater than that of seawater (~35 ‰), with some exceeding 100 ‰ (Glover 1982; McNeil *et al.* 2011b). Salinity increases as water losses occur (mostly through evaporation) and solutes concentrate (Hamilton *et al.* 2005; Sheldon *et al.* 2010). This may result in less-tolerant fishes being progressively excluded from waterbodies. A number of fishes are almost entirely restricted to fresh water in the LEB, including Australian smelt, Barcoo grunter, Cooper Creek catfish and golden goby, likely restricting their distribution (McNeil *et al.* 2015). In contrast, other LEB species can tolerate high salinities, even exceeding that of sea water. The Lake Eyre hardyhead can tolerate salinities of 110 ‰ (Glover 1982).

Fluctuations in oxygen levels, especially depressed levels or hypoxia, where less oxygen is available for aquatic respiration, is another challenge for LEB fishes. The effect of hypoxia is greater when temperatures are warmer, as oxygen demands are greater, but solubility of oxygen in water is lower (Tramer 1977). Hypoxia is often more extreme overnight, when aquatic vegetation is unable to photosynthesise and may become a net user of oxygen (McNeil & Closs 2007). Within the LEB, long-term isolation and stagnation of waterholes often results in hypoxia (McNeil *et al.* 2011b). Further, hypoxia has been suggested as a possible cause of fish-kills observed in the LEB, although low water temperatures have also been implicated (Jenny Davis, *pers. comm.*). Many fishes show adaptive responses to cope with low oxygen levels (Glover 1982). One such response is aquatic surface respiration, where fish ventilate their gills with oxygen-rich water from the air-water interface (Kramer & Mehegan 1981). A number of LEB fishes utilise this behaviour, including spangled perch, rainbowfish and desert goby (McNeil *et al.* 2011b).

While water quality frequently declines to severe levels in LEB waterholes, the conditions in Lake Eyre are even more extreme. Usually empty, Lake Eyre fills during large floods in the Basin, and is initially composed of fresh water and highly productive (Kotwicki 1986). A few fish species move into the lake and breed, with numbers estimated to reach 40 million individuals following the 1974 fill event (Ruello 1976). However, as inflows cease, the thick salt crust dissolves and the lake rapidly reaches high temperatures and salinity levels (Habeck-Fardy & Nanson 2014). This devastates fish populations. For example, thousands of dead Lake Eyre hardyhead and bony herring were observed stranded along the shorelines six months after the 1974 fill, and no live fish were found eight months later (Ruello 1976; Glover 1982). The extreme conditions of Lake Eyre, especially the high salinity, is thought to exclude many LEB fishes, with only five recorded from the Lake: Australian smelt, Lake Eyre hardyhead, spangled perch, golden perch, and bony herring (Unmack 2013).

The ability to tolerate severe water quality conditions is vital for persistence of fishes in the LEB. However, there are clearly differences in physiological tolerance levels among species (McNeil *et al.* 2015). This allows highly tolerant species to persist in many LEB waterbodies, while less-tolerant fishes are restricted to those waterholes that maintain relatively benign conditions. Because such waterholes are spatially and temporally variable, surviving in just these few habitat patches is unlikely to be a successful long-term strategy.

Dispersal Abilities

Where desert fishes cannot persist locally through drought periods, for example because conditions deteriorate beyond a species' tolerance or waterholes dry completely, then dispersal becomes essential for population persistence (Bogan *et al.* 2017). Dispersal allows fish to recolonise previously-dry habitats from refugial habitats when flows resume (Baguette *et al.* 2013). Many desert fishes are able to maintain populations only through dispersal, either from within the river system or from adjacent river systems (Chester *et al.* 2015). While opportunities for dispersal of aquatic taxa in deserts are limited, many desert fishes have strong dispersal abilities, generally greater than fishes in more mesic regions (Deacon & Minckley 1974; Kingsford 2006; Murphy *et al.* 2015; Bogan *et al.* 2017).

There is strong evidence for substantial dispersal of fish within LEB rivers. Numerous stories describe how anthropogenic habitats, such as bores, have been colonised by fishes, even when located many kilometres from known fish habitats (Wager & Unmack 2000). Observational studies confirm this, showing that LEB fishes can colonise newly-created habitats following flood events (e.g. McNeil & Schmarr 2009). In the Georgina-Diamantina River system, a previously dry tributary was recolonised by twelve fish species following serial flow events, with some fish dispersing up to 300 km (Kerezsy *et al.* 2013). Genetic studies provide another line of evidence, with multiple studies of LEB fishes showing minimal genetic structure within river systems, indicating gene flow among waterholes (e.g. Hughes & Hillyer 2006; Huey *et al.* 2008; Beheregaray & Attard 2015).

Desert fishes utilise multiple mechanisms for dispersal, which can be divided into active and passive dispersal. Drift, the downstream movement mediated by flow, is the most effective passive dispersal mechanism for fishes, and can transport eggs and larvae vast distances (Copp *et al.* 2002; King *et al.* 2005). Floods may also wash juveniles and adults downstream. Other passive mechanisms, such as phoresy (e.g. eggs attached to waterfowl legs), are unlikely to be important for dispersal of LEB fishes (Unmack 2001a; Worthington Wilmer *et al.* 2008). Dispersal via storm events, when fish are picked up by local wind storms and deposited some distance away, is also unlikely to be a common dispersal mechanism, although it has been frequently reported by LEB locals (Wager & Unmack 2000). Active dispersal, i.e. swimming, is the main way in which juvenile and adult fish disperse, and the only way fishes can move upstream. Many desert fishes are strong swimmers, able to

disperse tens or even hundreds of kilometres during flow events (Deacon & Minckley 1974; Kingsford 2006). While it is generally expected that larger species will have greater dispersal ability than smaller species (Jenkins *et al.* 2007), fishes do not follow this rule universally (Colvin *et al.* 2009; Shurin *et al.* 2009).

In the LEB, fishes are likely to disperse via both drift and swimming. Drift may be less likely to result in successful colonisation, given that it only operates in one direction, and downstream waterholes are typically rarer and more ephemeral than those in upper- to mid-reaches (Fensham *et al.* 2011). Further, a number of LEB fishes produce eggs that either are adhered to rocks or vegetation, or are demersal (sink into the substrate), and are therefore unlikely to be subject to drift (e.g. Glover 1971; Llewellyn 1973; Pusey *et al.* 2004). Some species do produce pelagic eggs however, and most have pelagic larvae, so some drift is likely for most fishes (Puckridge & Walker 1990; Pusey *et al.* 2004).

Active swimming should facilitate dispersal, but this has not been studied for LEB fishes, and it is unclear what traits are associated with active dispersal proclivity. However, observational studies indicate strong differences in the dispersal habits of different LEB fishes during floods. Kerezsy *et al*. (2013) categorised fishes in the Georgina-Diamantina River as either 'extreme' or 'conservative' dispersers. Extreme dispersers, including spangled perch, bony herring, silver tandan, rainbowfish and glassfish, rapidly disperse distantly and colonise all available habitats. In contrast, conservative fishes disperse more slowly and less distantly, and tend to colonise larger, more permanent, waterholes. Conservative dispersers, including hardyhead, golden goby, Hyrtl's tandan, barred grunter and golden perch, can travel significant distances (>100 km), but do not appear to disperse as readily, frequently or far as extreme dispersers (Kerezsy *et al.* 2013). Similar dispersal patterns were observed in the Neales River (McNeil *et al.* 2011b), suggesting that species' habits are consistent across the LEB.

In addition to biological determinants, dispersal is affected by environmental conditions. In aquatic systems, these include channel geomorphology and hydrology (Hughes *et al.* 2009). Within the LEB, rivers generally have shallow gradients and complex channels that allow flood waters to persist for relatively long periods of time, providing substantial opportunity for dispersal (Unmack 2001b). However, LEB rivers differ in flood frequency (Kotwicki &

Isdale 1991), and those that flow less often likely offer fewer dispersal opportunities. Rivers also differ in their geomorphology, e.g. the number of waterholes they contain (Fensham *et al.* 2011). Dispersal may be facilitated by the presence of waterholes, with fish more likely to be able to disperse between nearby waterholes than distant ones (Hughes *et al.* 2009).

Dispersal between LEB rivers seems to be less common than within rivers, with no known records of such movements. Further, most rivers have unique fish assemblages, indicating that some species have not been able to colonise other rivers in the LEB, despite occasional hydrological connections (Wager & Unmack 2000). This unusual distribution pattern has been attributed to Lake Eyre itself, which has been suggested to be a barrier to dispersal of LEB fishes (see above; Glover 1982; Unmack 2013). Only five of the LEB's fishes have been recorded in the ephemeral lake, one of which is restricted to a single river, while three others exhibit no evidence of contemporary gene flow among rivers (Masci *et al.* 2008; Faulks *et al.* 2010b; Huey *et al.* 2011a). While other fishes must be able to reach the lake, some may not be able to survive its extreme environment (Unmack 2013). In addition, dispersal can occur only during the short period when rivers flow to the lake, and requires two rivers to be flowing simultaneously, which likely occurs briefly and rarely, limiting dispersal opportunities. Lake Eyre may therefore inhibit dispersal between LEB rivers, at least for all but the most dispersive and environmentally-tolerant fishes.

Focal Taxa Review

In order to explore the evolutionary histories and futures of the LEB's fishes, seven species have been selected as focal taxa, from four families of fishes. These families are not closely related, having last shared a common ancestor at least 110 Mya (Betancur-R *et al.* 2013). The focal taxa represent most of the life histories and ecologies of LEB fishes, while also being present throughout most or all of the Basin (Wager & Unmack 2000). Three species are widespread throughout northern Australia, including the bony herring, spangled perch and barred grunter. The remainder are endemic to the LEB, and comprise two pairs of closely-related sympatric taxa: the desert goby and Finke goby, and the Lake Eyre hardyhead and Finke hardyhead. In Chapters 5 and 6, each of these species pairs is largely considered as a single taxon, which recent studies suggest may be a more accurate treatment (see following species accounts).

Here, the known biology of each taxon is reviewed, with particular focus on traits that are relevant to the current study. Unfortunately, there has been relatively little in the way of field studies of fishes in the LEB, with the exception of the desert goby that has been the subject of a significant body of research (initiated by the pioneering work of Glover 1971). Much of the understanding of these species' biology and ecology is derived from studies conducted on populations outside the LEB (bream, grunter, perch), or on related species (hardyhead). As such, the following descriptions are presented with the caveat that fishes in the extreme environment of the LEB may differ from populations elsewhere.

Desert & Finke Goby

The goby genus *Chlamydogobius* contains six species, of which five are endemic to the LEB (Larson 1995). Three are short-range species endemic to isolated spring systems, while the other two are more widespread and occur in both riverine and spring habitats. These include the desert goby, *Chlamydogobius eremius* (Zeitz, 1896), and the Finke goby, *Chlamydogobius japalpa* (Larson, 1995). These two gobies (Figs. 1–2) were differentiated based on the latter's disjunct distribution in the hydrologically-isolated Finke River and minor morphological differences in scalation patterns, fin ray counts and number of vertebrae (Larson 1995). More recent studies have indicated that the divergence between the two species is not as great as between populations of desert goby (Mossop *et al.* 2015). Accordingly, the two are treated as a single taxon here. The biology of the desert goby has been studied extensively in both field and laboratory studies (e.g. Glover 1971; Miller 1987; Michelangeli & Wong 2014; Moran *et al.* 2016; Mossop *et al.* 2017). The Finke goby is presumed to have a similar biology (Wager & Unmack 2000). Both species are small benthic fishes, which can grow to 60 mm in length.

None of the LEB *Chlamydogobius* gobies overlap in distribution, with three species restricted to the large Dalhousie, Edgbaston and Elizabeth Springs complexes (Larson 1995). The distribution of desert goby spans most of the rivers that connect to Lake Eyre, although it is rarely observed in the upper reaches of the Georgina-Diamantina and Cooper. It is also found in local spring systems, most of which connect to river systems during large rain events (Mossop *et al.* 2015). The Finke goby is restricted to the Finke River, and a small number of associated spring systems; it has been listed as threatened due to this restricted distribution (Northern Territory Government 2016). Both species share similar habitats,

including some anthropogenic habitats such as bores (Wager & Unmack 2000). They are often found in the shallow margins of aquatic habitats, and avoid the deeper water where other fishes are more abundant (Glover 1971). They frequently occur in very small pools, where extreme environmental conditions exclude most other fishes, including competitors and predators (Glover 1982; Mossop *et al.* 2017).

Figure 1. Adult male desert goby, *Chlamydogobius eremius*, showing brightly coloured fins that differentiate them from females and juveniles, which have clear fins. Photo Credit: Ashley Murphy.

Gobies are nocturnal, often hiding among macrophytes and detritus during the day (Glover 1971). They are omnivorous, and feed primarily on aquatic insects and microcrustaceans, supplemented with plankton, algae and detritus. Their long gut aids digestion of vegetation, and suggests they may be able to subsist on plant matter when nothing else is available (Miller 1987). They also take fish eggs (Glover 1971), likely non-buoyant eggs such as those of spangled perch and barred grunter. While fish scales were found in the stomachs of some desert gobies, these are likely from scavenging on dead fish (Glover 1971). Gobies face competition for food resources from the invasive mosquitofish or plague minnow, *Gambusia holbrooki*, which also attacks gobies and preys upon their eggs (Wager & Unmack 2000). Adult gobies are known to be preyed on by aquatic birds, and adults and juveniles may also be taken by predatory fishes, such as catfishes and perches. Smaller individuals may even be taken by predatory aquatic insects, such as beetle and dragonfly larvae (Glover 1971).

Desert goby are arguably the most environmentally tolerant fish in Australia, able to survive extreme fluctuations in water quality via physiological and behavioural adaptations (Glover 1971; Thompson & Withers 2002). The species inhabits water from 5 to 41 °C, and may survive short periods at even higher temperatures (Glover 1982). This temperature range approaches the upper biokinetic limit of ectothermic vertebrates that live in aquatic habitats (Brock 1985). Gobies can avoid extreme temperatures by retreating to cooler shaded or vegetated areas, burying themselves in cooler stream-bed silt, or emerging from the water to effect evaporative cooling (Glover 1971). The species is highly tolerant of salinity, with field experiments indicating that mortality of half of individuals occurred when salinities reached 52 ‰ (McNeil *et al.* 2011b). Some individuals, however, survived salinities in excess of 70 ‰, more than twice the salinity of seawater. Amongst LEB fishes, the salinity tolerance of gobies is second only to that of hardyhead (McNeil *et al.* 2015). Gobies also have exceptionally high tolerance of low oxygen levels, surviving short periods of extreme hypoxia that no other LEB fishes can (McNeil *et al.* 2011b). As oxygen levels fall, gobies utilise aquatic surface respiration and direct air-breathing, the latter adaptation unseen in other LEB fishes (Glover 1971; McNeil *et al.* 2011b).

The demographics of gobies are expected to be more stable than other LEB fishes, because they largely inhabit waterbodies in which they can persist permanently due to their environmental tolerances. However, when they colonise new habitats, population growth can be rapid (Glover 1971). Gobies mature within the first year, but rarely live beyond two years (Thompson 1983). Spawning occurs over summer, potentially cued by warmer temperatures, with two reproductive bouts common (Glover 1971). Males build nests under rocks or in crevices, and attract females by using elaborate courtship displays (Wong & Svensson 2009). The females lay small numbers (~50–250) of adhesive eggs that are deposited on the ceiling of the nest (Wager & Unmack 2000). As with other fishes, larger (likely older) females produce more eggs (Svensson *et al.* 2010). Males guard the eggs, which hatch after around ten days – there is no further parental care (Symons *et al.* 2011). Unlike other LEB fishes, goby larvae are not pelagic and instead sink to the substrate where development occurs (Mossop *et al.* 2015).

Figure 2. Adult male Finke goby, *Chlamydogobius japalpa*. Photo Credit: Michael Hammer.

Gobies have a poor swimming ability in comparison with other LEB fishes (McNeil & Schmarr 2009). However, the species' wide distribution and rapid colonisation of anthropogenic aquatic habitats suggest that it can disperse over long distances (Glover 1971). Mossop *et al.* (2015) demonstrated that gobies have unexpectedly high gene flow among populations, and that dispersal is sufficient to minimise genetic structure at the river system scale. Given this movement is unlikely to occur via larval drift, it has been suggested that transport of adults via floodwaters is the main dispersal mechanism (Glover 1982). Physiological tolerances, life history and individual behavioural traits may also increase the species' apparently limited dispersal potential (Mossop *et al.* 2015; Mossop *et al.* 2017).

Lake Eyre & Finke Hardyhead

The hardyhead genus *Craterocephalus* contains 25 species native to Australia and New Guinea, of which five are native to the LEB. Of these, the Lake Eyre hardyhead, *Craterocephalus eyresii* (Steindachner, 1883), is the most widespread. As with the gobies, the other species include short-range spring endemics and a species restricted to the isolated Finke River: the Finke hardyhead, *Craterocephalus centralis* (Crowley & Ivantsoff 1990). The Lake Eyre and Finke hardyheads are sister species, separated largely by modal differences in minor morphological features, including scalation patterns, fin ray counts and aspects of bone shape (Crowley & Ivantsoff 1990). These two species are practically morphologically indistinguishable in the field (Wager & Unmack 2000), and recent phylogenetic studies indicate that the two species are not genetically distinct (Unmack &

Dowling 2010; Adams *et al.* 2011). Given their similarity, the two hardyhead species are treated as a single taxon in this thesis. Little is known about the Finke hardyhead, but it is likely very similar to the Lake Eyre hardyhead (Wager & Unmack 2000). Both are long, narrow fishes, which can grow to 100 mm in length, although are often around 40–70 mm (Wager & Unmack 2000).

Figure 3. Finke hardyhead, *Craterocephalus centralis*, collected in the Finke River. Photo Credit: Michael Hammer.

The Lake Eyre hardyhead is endemic to the LEB, where it inhabits most river systems that connect to Lake Eyre, although is rarely found in the Georgina-Diamantina and Cooper river systems (Kerezsy *et al.* 2013). It is one of five fishes recorded from Lake Eyre itself (Glover 1982). The Finke hardyhead is endemic to the isolated Finke River, and is considered threatened due to its restricted distribution (Northern Territory Government 2016). Both species inhabit riverine waterholes, spring systems and anthropogenic habitats. Two other hardyhead species are endemic to the Dalhousie Springs complex (Ivantsoff & Glover 1974; Crowley & Ivantsoff 1990); these species are distantly-related to the Lake Eyre and Finke hardyheads and descend from a lineage that colonised the LEB at a different time (Unmack & Dowling 2010). The final species, the fly-specked hardyhead, is recorded from the upper Cooper Creek and in the far south of the Basin, and is also present in other drainages in northern Australia (Hammer & Walker 2004). The range of this species overlaps with that of the Lake Eyre hardyhead, although it is not closely related and may represent a third colonisation of the LEB by the genus (Unmack & Dowling 2010).

Lake Eyre and Finke hardyhead are highly tolerant desert specialists, which are abundant in particularly warm, shallow and saline waterbodies (McNeil *et al.* 2011b). The salinity tolerance of Lake Eyre hardyhead is higher than that of any other Australian fish, with individuals surviving salinities up to 110 ‰ (Wager & Unmack 2000; McNeil *et al.* 2015). This tolerance allowed them to thrive in Lake Eyre when it filled in 1974, and reach an abundance of ~20 million, before conditions ultimately became too saline as the lake dried and all fish were killed (Ruello 1976). Hardyhead are also tolerant of a wide range of temperatures, and exist in water from 9 to 37 °C (Glover 1982). It is possible that individuals can survive a wider range; the Dalhousie hardyhead survives temperatures exceeding 40 °C (Glover 1989). Hardyhead appear to be less tolerant of hypoxia however, and are less tolerant of depressed oxygen levels than most LEB fishes (Glover 1982).

Hardyhead are shoaling, pelagic species that feed mostly in the water column. Their diet consists of filamentous algae and aquatic invertebrates, primarily microcrustaceans (Wager & Unmack 2000). While hardyhead share a similar diet with gobies and are often found in the same habitats, they may not be direct competitors; because hardyhead are pelagic and gobies benthic (Glover 1971). Hardyhead are an important food resource for waterbirds and larger fishes, likely including spangled perch and golden perch in the LEB (Kennard 1995; Allen *et al.* 2002).

Individuals reach maturity within their first year, and are thought to rarely live for a second year (McNeil *et al.* 2011b). Reproduction occurs primarily during the warmer months, but the species may also opportunistically breed during floods, which allows rapid population growth during boom periods (Allen *et al.* 2002). During a protracted spawning season, hardyhead lay small numbers of eggs daily, for a total seasonal fecundity averaging ~200 eggs (Unmack 1995; Hammer & Wedderburn 2008; McNeil *et al.* 2011b). The eggs are fixed to aquatic vegetation with adhesive threads, and there is no egg-guarding or parental care (Allen *et al.* 2002). Eggs hatch in five to ten days, and the free-swimming larvae mature rapidly (Hammer & Wedderburn 2008).

Whilst hardyhead are relatively small fishes, they show evidence of strong dispersal capabilities (Hammer & Wedderburn 2008). For example, Lake Eyre hardyhead were observed to colonise a previously dry tributary in the Georgina-Diamantina River, at least

300 km from existing populations, following flow resumption (Kerezsy *et al.* 2013). This indicates that the species is capable of long-distance dispersal. However, they took several years following the initial hydrological connection to make this movement, suggesting that they may be relatively conservative dispersers (Kerezsy *et al.* 2013). A similar pattern was observed following flooding in the Neales River, where the species dispersed to new habitats more slowly than did most other fishes (McNeil *et al.* 2011b).

Barred Grunter

The banded or barred grunter, *Amniataba percoides* (Günther, 1864), is an Australian endemic member of the Terapontidae, one of the continent's more speciose fish families. The genus *Amniataba* includes two other species; one freshwater species endemic to New Guinea, and one marine species found in coastal waters of northern Australia and New Guinea (Pusey *et al.* 2004). Barred grunter are a small, aggressive fish, which can grow to 200 mm long, but more often only attain 120 mm (Wager & Unmack 2000). They are light brown in colour, with striking dark stripes (Fig. 4). They form loose shoals throughout their wide range, but are usually only present in small numbers.

Figure 4. Barred grunter, *Amniataba percoides*, collected in the Finke River and showing the species' distinguishing bold vertical bars. Photo Credit: Michael Hammer.

Barred grunter are widely yet patchily distributed across the coastal drainages of northern Australia, as well as the LEB. It is absent from the adjacent Bulloo and Murray-Darling basins, unlike most other widespread inland fishes (Pusey *et al.* 2004). In the LEB, grunter

are found in most rivers, although they are notably absent from Cooper Creek (Wager & Unmack 2000). Grunter occur mostly in riverine waterholes, and less commonly in springs or anthropogenic aquatic habitats. The species is benthic, with adults typically found in deeper waters than juveniles (Bishop *et al.* 2001).

Grunters are generalists that feed on a wide range of food sources. Their flexible diets allow them to take advantage of locally abundant resources, and compete with other terapontids (Pusey *et al.* 2004). Barred grunters are benthic foragers, and feed predominantly on aquatic insect larvae, molluscs and microcrustaceans. Adults feed more heavily on aquatic macrophytes and algae than do smaller fish, and may also take larger macrocrustaceans, such as shrimp (Bishop *et al.* 2001). Fish make up a small part of the species' diet. In some tropical rivers this includes species of hardyhead (Bishop *et al.* 2001); it is unknown if they feed on hardyhead in the LEB. The species will also scavenge, and take terrestrial invertebrates, especially when on the floodplain (Bishop *et al.* 1995; Wager & Unmack 2000).

The demography and reproductive biology of barred grunters is poorly known, and has not been studied in the LEB. The species likely experiences substantial variation in population size, but is generally found in relatively small numbers (Wager & Unmack 2000). Grunter reach sexual maturity within their first year, and may live three to four years (Pusey *et al.* 2004). Spawning is not triggered by flooding, but occurs largely over the warmer months, suggesting it may be cued by temperature or day length (Pusey *et al.* 2004). Females produce around 125,000 eggs (maximum recorded = 400,000 eggs), released into the water column to sink to the substrate (Bishop *et al.* 2001). Females may produce multiple clutches per breeding season. There is no parental care and eggs hatch within a few days, likely followed by rapid larval development (Wager & Unmack 2000).

Barred grunter show a range of movements through river systems, with most dispersal undertaken by juveniles (Pusey *et al.* 2004). One study in tropical Queensland found adults and juveniles moving upstream towards refugial waterholes at the start of the dry season (Bishop *et al.* 2001). Adults were found to swim around 9 km per day, while juveniles swam around 7 km, with both moving only during daylight hours (Bishop *et al.* 1995). Within the LEB's Georgina River, barred grunter was found to be a conservative disperser, colonising new habitats more slowly than most other fishes (Kerezsy *et al.* 2013).

Little is known about the environmental tolerances of barred grunter, although some inferences have been made. Bishop *et al.* (2001) suggest that they can likely tolerate similar temperatures to spangled perch, another terapontid, based on their similar distributions. This suggests grunter can tolerate temperatures of ~10–35 °C (Pusey *et al.* 2004). Grunter appear tolerant of elevated salinity, with some surviving salinity levels in excess of 50 ‰ (McNeil *et al.* 2015). However, an experimental study of barred grunter in the LEB's Neales River showed that a salinity of 21 ‰ was sufficient to cause mortality in half of exposed fish (McNeil *et al.* 2011b). They are also moderately tolerant of hypoxia, with small numbers observed in some rivers with low oxygen levels (Pusey *et al.* 2004).

Spangled Perch

The spangled perch, *Leiopotherapon unicolor* (Günther, 1859), is an Australian endemic and the continent's most widespread freshwater fish species. Spangled perch are Terapontidae, as are barred grunter; their genera likely diverged ~15 Mya (Peter Unmack, *pers. comm.*). Spangled perch belong to a small genus that contains three other species, one endemic to the Philippines and two short-range endemics of north-west Australia. Spangled perch are medium-sized omnivores, potentially growing to 300 mm, but more often only reaching 150 mm. They are silvery in colour, with bluey-brown or bronze speckles (Wager & Unmack 2000). This aggressive fish lives in small shoals, and is abundant and ubiquitous throughout the LEB's waterbodies.

Figure 5. Spangled perch, *Leiopotherapon unicolor*. Photo Credit: Ross Felix.

Spangled perch are found across northern Australia, from the Pilbara to Cape York, and throughout the internal drainages of the Western Plateau, and the Lake Eyre, Bulloo and Murray-Darling basins. It is thought that the species' southern distribution is limited by lower water temperatures (Pusey *et al.* 2004). Within the LEB they are the most widespread fish, found in every river system, including a number of isolated rivers, such as the Todd, Plenty and Hale, that lack other fishes (Unmack 2001b). They are found in a wide variety of aquatic habitats, from riverine waterholes to large spring systems, as well as all types of anthropogenic aquatic habitats, even water troughs (Wager & Unmack 2000).

Spangled perch have a flexible, generalist diet, and show ontogenetic dietary variation, which may reduce interspecific competition (Pusey *et al.* 2004). Invertebrates, primarily aquatic insects and macrocrustaceans, are the primary food resource, and are almost the only food consumed by juveniles. Terrestrial invertebrates and detritus are also taken, especially when perch move onto the floodplain (Kennard 1995). As with barred grunter, perch supplement their diet with aquatic vegetation, with larger fish feeding more heavily on macrophytes (Pusey *et al.* 2004). Perch, especially larger adults, also feed on other fishes. This predation plays a role in structuring fish communities in a tropical Queensland river (Kennard 1995).

Spangled perch have excellent dispersal abilities, and are arguably the most dispersive Australian fish. One early report details hundreds of juvenile perch swimming 16 km over a six hour period along a flooded wheel rut, including through shallow sections where their backs were out of the water (Shipway 1947). The species is frequently reported to have been picked up by storms and dropped far from water, in "rains of fishes" (Unmack 2001b; Pusey *et al.* 2004). However, the presence of perch far from water and their sudden appearance in new pools can be explained by dispersal through shallow overland flows during rain events (Unmack 2001b). Exceptional dispersal abilities also explain their presence in almost all aquatic habitats (Wager & Unmack 2000). Dispersal from refugial habitats appears to be triggered by flooding, facilitating colonisation of newly available habitat (Pusey *et al.* 2004). For example, following floods in the LEB, spangled perch rapidly dispersed over 300 km and inhabited all habitat patches in a previously dry tributary (Kerezsy *et al.* 2013).

Spangled perch are abundant, frequently exhibiting rapid population growth, especially following colonisation of new habitat. They mature within their first year, and may live four to five years (Pusey *et al.* 2004). Spawning occurs in large aggregations, usually in summer, apparently cued by temperature rather than flooding (Llewellyn 1973). However, in the ephemeral waterbodies of the LEB, spawning may be more opportunistic (Bostock 2014). Spangled perch are highly fecund; females produce tens of thousands of eggs, with a mean of ~48,000 in one northern river, and a maximum of ~113,000 recorded (Llewellyn 1973). Eggs are released into the water, where they sink into the substrate and hatch within a few days. There is no parental care. Larval development takes around a month, and the juveniles grow rapidly (Llewellyn 1973). As with most LEB fishes, recruitment is greater when spawning coincides with floods, which increase habitat and food availability (Pusey *et al.* 2004).

Tolerance of a wide range of environmental conditions allows spangled perch to inhabit diverse habitats (Pusey *et al.* 2004). Spangled perch can tolerate a wider temperature range than all other studied LEB fishes, surviving temperatures of 4.1–45.0 °C (Llewellyn 1973; Glover 1989). Perch can tolerate moderate salinities, with an experimental study in the LEB finding that mortality of half of individuals was caused by salinity levels of 15 ‰, and complete mortality occurring at 30 ‰ (McNeil *et al.* 2011b). Spangled perch have large respiratory surfaces, allowing them to survive low oxygen levels (Pusey *et al.* 2004). In hypoxic waters, perch are commonly found near the surface, where they utilise aquatic surface respiration to increase their oxygen intake (McNeil *et al.* 2011b). They also attempt 'escape behaviours', such as jumping, which may be an attempt to disperse away from extreme conditions (McNeil *et al.* 2011b).

Bony Herring

The bony herring or bony bream, *Nematalosa erebi* (Günther, 1868), is the only member of the herring family found in the LEB. It is endemic to Australia, and has the second widest distribution of any freshwater fish on the continent (Wager & Unmack 2000). Bony herring are relatively large fish, and may reach lengths of 450 mm, although are typically 150–300 mm (Pusey *et al.* 2004). They are bright silvery-white, with small scales and clear fins (Fig. 6). Herring are a pelagic, shoaling fish that is generally very abundant where present (Wager & Unmack 2000).

The distribution of bony herring covers much of coastal northern Australia, from the west coast to the east coast, as well as the interior Lake Eyre, Bulloo and Murray-Darling basins (Pusey *et al.* 2004). Within the LEB, the species is found in all major rivers, and is primarily found in waterholes and occasionally bores, and rarely in springs (Wager & Unmack 2000). Bony herring are known to inhabit the ephemeral salt lake habitat provided by the filling of Lake Eyre (Glover 1982).

Adult bony herring are primarily herbivorous, while juveniles are omnivorous (Wager & Unmack 2000). Adults feed mostly on detritus and algae, although one study in the LEB found a third of their diet consisted of plankton, aquatic insects and small molluscs (Bishop *et al.* 2001; Pusey *et al.* 2004). Gut content analyses of adults in Lake Eyre showed herring were feeding mostly on invertebrates – almost entirely ostracods and chironomid larvae (Ruello 1976). During flood periods, herring move onto the floodplain, where adults feed mostly on terrestrial detritus and juveniles on invertebrates (Balcombe *et al.* 2005). Herring are therefore an important part of the LEB aquatic community; their consumption by higher trophic level consumers facilitates rapid transfer of aquatic and terrestrial primary production through the aquatic food web (Pusey *et al.* 2004).

Figure 6. Bony herring, *Nematalosa erebi*, collected in the Finke River. Photo Credit: Michael Hammer.

The species shows strong 'boom and bust' demographics, with breeding occurring throughout spring and summer, independently of flooding (Wager & Unmack 2000). Herring mature early, usually in their first year, and may live up to five years (Pusey *et al.* 2004). Spawning occurs in shallow waters where herring congregate in large numbers; buoyant eggs are released and fertilised in the water (Puckridge & Drewien 1988; Bishop *et al.* 2001). Bony herring are highly fecund, with females producing up to 880,000 eggs per spawning event (Puckridge & Walker 1990). There is no parental care and larval development is rapid (Pusey *et al.* 2004). Recruitment is likely to be more successful when spawning coincides with flooding (Puckridge & Walker 1990). The species is often highly abundant, and dominates the fish biomass of many LEB rivers during summer (McNeil *et al.* 2011b).

Bony herring are strong dispersers that rapidly migrate following flood events (Pusey *et al.* 2004). For example, herring soon dispersed to newly-filled waterholes at least 300 km from existing habitat following a flood in the LEB's Georgina-Diamantina River (Kerezsy *et al.* 2013). Movements in other regions appear to be undertaken primarily by juvenile fish, upstream, with adult herring appearing less vagile than other fishes (Russell 1991; Bishop *et al.* 2001; Marshall *et al.* 2016). Such rapid and distant dispersal allows the species to take advantage of newly available habitat, which (at least initially) lacks competitors and predatory fishes, and may confer a recruitment advantage (Kerezsy *et al.* 2013).

While tolerant of a wide range of environmental conditions, herring are not as tolerant as desert specialists. Herring seem especially intolerant of temperature extremes, and in the LEB are found only in waters of 14–30 °C (Glover 1982). Mass kills of herring frequently occur in winter, attributed to low temperatures associated with increased rates of parasitic and fungal infections (Langdon *et al.* 1985). Herring can tolerate salinities of at least 39 ‰, and some observations suggest they may be able to tolerate over 50 ‰ (Ruello 1976; Glover 1982). They are intolerant of hypoxia, and become inactive in such conditions (Pusey *et al.* 2004). Bony herring appear to be easily stressed and sensitive, with experimental studies in the LEB abandoned following high mortality in captivity (McNeil *et al.* 2011b).

The Persistence Ecology of the Lake Eyre Basin Fishes

Persistence in extreme environments often requires species to maintain multiple populations, so that local extirpation events do not result in species' extinction. This is certainly true for the fishes of the LEB. As none of the Basin's rivers have permanent flowing water, the longest lasting fish habitats are large, relatively permanent waterholes, although even these may become uninhabitable for fish, for example when they become too saline or dry completely (Chapter Three). For fishes to persist in the LEB then, not all waterholes can be uninhabitable at the same time, and fishes must maintain populations in multiple waterholes (Unmack 2001b). A variety of life history traits can contribute to this persistence, including environmental tolerances and dispersal abilities. Here, suites of such traits are termed 'persistence strategies'.

Many desert aquatic taxa exhibit persistence strategies, which exist along a continuum from resistance to resilience (Bogan *et al.* 2017). Resistance strategies allow populations to persist *in situ* through disturbance events, while resilience strategies allow repopulation following a disturbance event via recolonisation from *ex situ* habitats (Lake 2000; Nimmo *et al.* 2015). In general, most desert fishes tend to be widespread resilience-strategists that persist primarily via dispersal (Kerezsy *et al.* 2017). These fishes exhibit traits that facilitate maintenance of populations in larger, environmentally-benign waterholes during drought, and recolonisation following flow resumption (Bogan *et al.* 2017). Fewer fishes are resistance-strategists; these tend to be desert specialists with adaptations that allow persistence in extreme habitats during drought (Kerezsy *et al.* 2017).

Within the LEB, one study has explored fish persistence strategies, through observations of the responses of different species to drought and subsequent floods (McNeil *et al.* 2011b). This study was undertaken in the Neales River following a severe drought in 2006–2007, and investigated the nine fishes present, including the five taxa studied here (excluding the Finke endemics). During the drought, a small number of aquatic refuges persisted, including one large waterhole, and several smaller waterholes with more extreme conditions, notably high salinity (McNeil & Schmarr 2009). While all nine fishes were present in the large 'ark' refuge, only the two most environmentally-tolerant fishes (desert goby and Lake Eyre hardyhead) persisted in the smaller 'polo club' refugia (Robson *et al.* 2008; McNeil *et al.* 2011b).

In late 2007, a catchment-wide flood briefly restored hydrological connectivity throughout the Neales River (McNeil & Schmarr 2009). This refilled a number of waterholes, which were rapidly colonised by spangled perch and bony herring. None of the other species were found to have dispersed to new sites during this flow period. The next flood, in late 2008, was of a longer duration, and during this flood the desert rainbowfish and golden perch colonised all available waterholes (McNeil & Schmarr 2009). The Lake Eyre hardyhead also colonised a number of sites, but did not disperse as far as the other fishes. The remaining fishes (desert goby, barred grunter, Barcoo grunter and invasive plague minnow) did not disperse beyond the site/s they inhabited during the drought, although had recolonised some new sites by 2010 (McNeil *et al.* 2011b).

The recolonisation responses of these species were used by McNeill *et al*. (2011) to determine their persistence strategies (Table 2). Of the focal taxa of this study, two are resilience-strategists (bony herring and spangled perch), two are resistance-strategists (desert goby and Lake Eyre hardyhead, and their sister-species in the Finke River), and one appears to have an intermediate strategy (barred grunter). Post-drought recolonisation by LEB fishes have since been observed in the Georgina River, with similar responses reported for all species (Kerezsy *et al.* 2013).

Table 2. Life history traits, including relative longevity, fecundity, dispersal ability and environmental tolerance, and predicted reproductive and persistence strategies of the five study species (modified from McNeil *et al.* 2011b). For specific details of life history traits, see above species' accounts.

Taxa	Longevity	Fecundity	Reproductive Strategy	Dispersal Ability	Environmental Tolerance	Persistence Strategy
Goby	Short	Low	Equilibrium	Weak	High	Resistance
Hardyhead	Short	Low	Opportunistic	Moderate	High	Resistance
Barred Grunter	Long	Moderate	Opportunistic	Weak	Low	Intermediate
Spangled Perch	Long	High	Opportunistic	Strong	Low	Resilience
Bony Herring	Long	High	Opportunistic	Strong	Low	Resilience

Anthropogenic Influences on the Lake Eyre Basin Fishes

Humans have had a wide range of impacts on the fishes of the LEB. While the arrival of Aboriginal people in the LEB at least 30,000 years BP (Thorley 1998) is not expected to have major impacts on the region's fish fauna, the arrival of European settlers in the mid-19th Century certainly did. These impacts are primarily through alterations, including loss and degradation, of aquatic habitats, and reduced connectivity between them, although these impacts are less strong than in other parts of Australia and in other arid regions (Kingsford *et al.* 2014; see Chapter Three for further details). In addition to environmental influences, humans can also directly affect LEB fishes, for example through fishing.

Recreational fishing is a popular activity in the LEB, with 400 anglers partaking in the activity in the 2007/2008 year (PIRSA 2013). The most commonly taken species is Lake Eyre golden perch, the largest LEB fish, with terapontids and catfish also targeted (PIRSA 2013). A number of fish species are fully protected, especially short-range endemic species, although this varies among states/territories (PIRSA 2013; QDAF 2015). The direct impacts of recreational fishing are minimal for most LEB fish species, although reduction of populations of long-lived species, such as golden perch and Cooper catfish, in refugia during drought periods may threaten their ability to persist (PIRSA 2013).

Aboriginal groups also fish across the LEB, for sustenance, social and cultural reasons (Kimber 1984). Spangled perch are believed to be primary target species for indigenous communities, and is likely an important subsistence fishery in the region (PIRSA 2013). Bony herring are also likely to be important in some regions (Jenny Davis, *pers. comm.*). While it is not known how many fish are taken by indigenous communities, the impact on most species is expected to be minimal, with spangled perch being extremely abundant and widespread (McNeil *et al.* 2011b).

Commercial fishing is generally not permitted (and unlikely to be profitable) in the LEB, with only one venture licensed. This is for the taking of 350 tonnes per fishing event of Lake Eyre golden perch from two lakes in the South Australian portion of Cooper Creek (PIRSA 2015). Barcoo grunter and Welch's grunter may be taken as by-catch. Fishing is only permitted after these lakes become disconnected from the main channel as Cooper Creek flows cease. Poor water quality will eventually kill all fish in the isolated lakes as they dry

(PIRSA 2015). As such, the impact on these species is likely to be minimal, especially given the lakes have filled only twice since the practice was first permitted in 1992 (PIRSA 2013).

Another way humans can influence species is by translocation (Simberloff *et al.* 1998; Lintermans 2004). There is no evidence for fish translocations by Aboriginal people in the LEB, although this has been documented in other parts of Australia (Pascoe 2015), and at least one plant species may have been translocated over 1000 km to the LEB around 15,000 years BP (Kondo *et al.* 2012). While historic translocation of aquatic animals across the central Australian arid region seems extremely unlikely, it cannot be discounted.

Recently, the number of translocations has increased greatly (Lintermans 2004). This has resulted in the introduction of a number of fishes not native to the LEB, but also of native species to areas they have not previously been recorded in, or where they have become locally extinct. For example, during his research in the South Australian portion of the LEB, John Glover translocated desert gobies to a number of springs where the species was not previously known to occur (Glover 1971). While researchers would not (hopefully) unnecessarily translocate fishes today, anglers (and others) are believed to re-stock waterholes frequently, especially with spangled perch (Wager & Unmack 2000; Kerezsy 2017).

A number of Australian fishes not native to the LEB have also been introduced, most for angling purposes (Wager & Unmack 2000). The Murray cod, *Maccullochella peelii*, was stocked in the Cooper, while the Murray-Darling golden perch, *Macquaria ambigua ambigua*, was introduced to several locations (Wager & Unmack 2000). Silver perch, *Bidyanus bidyanus*, was introduced near Lake Eyre (Wager & Unmack 2000). As these fishes do not appear to have persisted, they are unlikely to present a serious threat. One threat posed by the introduced Murray-Darling golden perch and silver perch is through hybridisation with native congenerics, the Lake Eyre golden perch and Welch's grunter respectively, the former an endemic species (Wager & Unmack 2000). Hybridisation from fish stocking has led to negative outcomes for some fishes globally and in Australia, including loss of diversity and outbreeding depression (Ford 2002; Nock *et al.* 2011). However, without continued stocking, it is unlikely that this will have occurred within the LEB. The sleepy cod, *Oxyeleotris lineolata*, is a recent introduction that has rapidly

colonised most of the Cooper Creek drainage since its first recorded sighting in 2008 (Kerezsy 2017). The impacts of this species are unknown, but it is a large carnivorous fish, and there are concerns for smaller fishes and the short-range endemic Cooper Creek catfish (Kerezsy 2017). Given its popularity among anglers, there is a risk that sleepy cod may be spread further through the LEB.

While most exotic species have not persisted, including the one-spot livebearer *Phalloceros caudimaculatus*, swordtail *Xiphophorus helleri* and carp *Cyprinus carpio*, two others have established breeding populations. Of greatest concern is the plague minnow (Fig. 7), which is abundant in the Neales, Cooper and Diamantina, and spreading (McNeil *et al.* 2011b). This species poses a significant threat to native LEB fishes, and has been implicated in the decline of many species globally following widespread introductions (Lloyd 1990). It is a small but aggressive fish, that is likely to especially threaten other smaller fish species, such as gobies and hardyhead, via competition and predation on eggs and juveniles (Arthington & McKenzie 1997; McNeil *et al.* 2011b). These impacts may be reduced in riverine systems where the extreme boom and bust conditions may limit plague minnow populations, but are likely to be severe in the less-variable conditions of springs (Wager & Unmack 2000; Fensham *et al.* 2011).

Figure 7. Abundant invasive mosquitofish or plague minnow, *Gambusia holbrooki*, among aquatic vegetation at Algebuckina Waterhole, Neales River, South Australia. Photo Credit: Ashley Murphy.

Goldfish, *Carassius auratus*, are also present, mostly in Cooper Creek (Wager & Unmack 2000). This fish is unlikely to have much of an impact on native fish species, given its' current restricted range and relatively small population size. However, it is increasing in abundance and is a broad generalist that may compete with other species, feed on their eggs, reduce aquatic vegetation and increase turbidity (Arthington & McKenzie 1997; Morgan & Beatty 2007; Costelloe *et al.* 2010).

Introductions of other freshwater animals have also occurred in the LEB, primarily of Australian species. While one turtle species occurs naturally in the LEB, the Cooper Creek turtle, *Emydura macquarii emmotti*, other Australian and exotic species have been found in other parts of the LEB, including the Finke River (Georges & Thomson 2010). These are generally thought to be dumped pets and have been removed where found (Jayne Brim Box, *pers*. *comm*.). As such, their impacts on native fishes are likely minimal. The recent introduction and subsequent range expansion of the farmed red-claw crayfish, *Cherax quadricarinatus*, from northern Australia, is expected to have major impacts on the native blue-claw crayfish or yabby, *C. destructor*, and potentially also impact fishes (Woodford 2008; Kingsford *et al.* 2014; Kerezsy 2017).

Figure 8. Red-claw crayfish, *Cherax quadricarinatus*, caught in a net along with large numbers of juvenile spangled perch, *Leiopotherapon unicolor*, at Eringa Waterhole, Macumba River, South Australia. Photo Credit: Ashley Murphy.

A number of fish species within the Lake Eyre Basin are listed as threatened under the Commonwealth Environment Protection and Biodiversity Conservation Act 1999 and/or on state-based lists (Lintermans 2013). All listed fishes are short-range endemics, many of which do not face current threats and their listing reflects their vulnerability arising from naturally small distributions. This includes the Finke hardyhead and Finke goby, which are restricted to the Finke River, but face no direct threats (Northern Territory Government 2016). Other species illustrate directly how vulnerable such populations can be. For example, the endangered red-fin blue-eye is found only in the Edgbaston spring complex in the north-east of the LEB and is rapidly declining as populations are extirpated by the introduced plague minnow, likely exacerbated by habitat degradation from introduced hoofstock (Fairfax *et al.* 2007; Kerezsy & Fensham 2013). The blue-eye's fellow local endemic, the Edgbaston goby, is similarly threatened (Faulks *et al.* 2017). In general, exotic species seem to be the most pressing direct threat to LEB fishes, especially those with small distributions, with other anthropogenic impacts less severe.

It is unclear how future climate change will affect LEB fish, but Australian fishes in arid regions are expected to experience greater negative impacts than those in wetter areas (James *et al.* 2017). As discussed in Chapter Three, climate change modelling suggests that the LEB will experience warmer temperatures, changes in rainfall patterns (with a decrease in precipitation levels across much of the LEB) and more frequent drought events (Watterson *et al.* 2015), which will reduce hydrological connectivity and the number of habitats that persist between flow events. Water extraction may also increase, as anthropogenic demand for water increases across Australia (Kerezsy *et al.* 2017). The fishes threatened most by climate change are those that are most sensitive to environmental extremes, but all species will be negatively affected if the number of habitats that provide refuge through drought periods decreases in the future (Filipe *et al.* 2013).

Conclusion

Despite the extreme environment of the LEB, it is home to a surprising diversity of freshwater fishes that represent a range of different taxonomic families. These fishes are evolutionarily distinct from fishes of deserts in other parts of the world, due to the derivation of most Australian fishes from otherwise marine families (Humphries & Walker 2013).

However, they share many of the same challenges faced by other desert fishes, and exhibit a similar range of life history traits. In general, they are more dispersive and tolerant of environmental extremes than fishes in mesic regions, although there are significant differences in the strength of these traits among LEB fishes. These differences are thought to contribute to different approaches to persistence, with the utilisation of strategies that range from resistance to resilience.

The implications of these different persistence strategies for the evolution of LEB fishes has not been investigated in detail. Thus, there are significant knowledge gaps regarding how species ecology has affected, and will affect in the future, the evolution of these fishes. For example, it is unknown how ecology may influence the ability of species to maintain genetic diversity, which is vital for the maintenance of long-term evolutionary potential. It is also unclear how ecological traits may influence the evolutionary trajectories of isolated fish populations, which is a significant issue in regions with low hydrological connectivity. An understanding of how these processes operate for LEB fishes will be applicable to other desert fishes globally, and potentially to other taxa and ecosystems.

To investigate these knowledge gaps, seven species from the fish fauna of the LEB have been selected as study taxa for this project. In Chapters Five and Six, a population genomic-based exploration of these species' evolutionary histories, contemporary population processes and ability to adapt to future environmental change is conducted. These fishes were chosen as they represent a range of ecologies, from widespread generalists to desert specialists, and cover the full range of persistence strategies observed in the LEB. In addition, they are widespread throughout most or all of the LEB's river systems, allowing for detailed comparisons across a wide spatial scale. It is expected that the exploration of influence of these species' ecology on their evolution will have direct implications for their management, as all face future risks from climate change and other anthropogenic impacts. The management implications are likely to also be applicable to fishes with similar ecologies in deserts globally.

Chapter Five

High and Dry:
Consequences of Alternative Persistence Strategies for the Population Connectivity of Desert Fishes

Abstract

Persistence of biodiversity in highly fragmented habitats often relies on metapopulation dynamics, which can be strongly influenced by spatial and temporal transience of habitat patches. For desert fishes in the Lake Eyre Basin of central Australia, persistence relies on their ability to maintain population connectivity across isolated waterholes within and among ephemeral river systems. To persist here, fishes possess different suites of traits (persistence strategies), which range from resistance (high tolerance of environmental extremes, but low mobility) to resilience (the converse). To test the hypothesis that different persistence strategies lead to differences in population connectivity, a novel population genomics approach, MetaPrep, was used to compare the population structure of five fish taxa across the Lake Eyre Basin. MetaPrep samples anonymous nuclear loci across the genomes of multiple species simultaneously, and here yielded a dataset of >700 nuclear DNA sequences for each taxon. The results showed that, within rivers, resilient taxa exhibited high population connectivity, while resistant taxa exhibited both high and low population connectivity. Among rivers, population connectivity was lower, but followed similar patterns amongst strategies. However, at this scale, population connectivity was more strongly influenced by structural connectivity, including hydrological connections and environmental variables. Overall, resilient and resistant strategists were both effective at maintaining population connectivity in this extreme and fragmented desert river system. Future persistence is likely to be similar among resistant and resilient taxa, although the latter are likely to be less able to respond to future environmental changes if structural connectivity is lost. Persistence strategies may provide an effective tool to characterise the population connectivity, contemporary and future persistence, and appropriate management scales for desert fishes.

Introduction

In many highly-fragmented environments, persistence of species is often affected by the spatial and/or temporal transience of habitat patches. Taxa in these environments often operate in a metapopulation structure, with spatially separated subpopulations occupying some or all available habitat patches, each susceptible to local extinction (Hanski 1999; Fagan 2002). In order for metapopulations to persist, individuals must disperse between patches when possible, maintaining subpopulations in multiple patches and facilitating recolonisation following local extinction (Fagan 2002; Mari *et al.* 2014; Sousa-Santos *et al.* 2014). Recolonisation is dependent upon population connectivity, which comprises genetic connectivity, determined by the effects of gene flow on evolutionary processes, and demographic connectivity, determined by the effects of migrants on the growth rates of subpopulations (Lowe & Allendorf 2010).

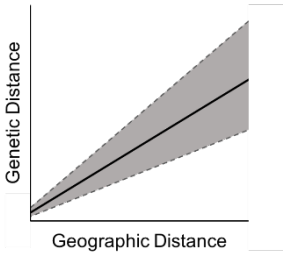
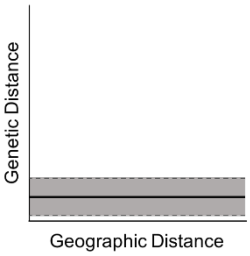
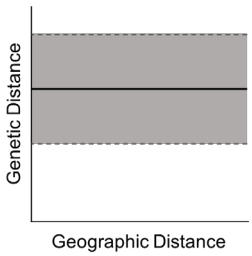
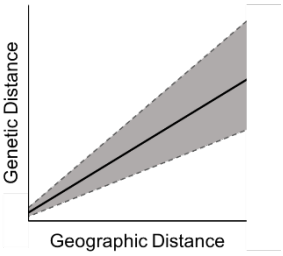
In addition to facilitating metapopulation persistence through recolonisation, population connectivity is often vital for the maintenance of a species' adaptive potential: the ability to adapt to a changing environment, partially driven by genetic diversity (Willi *et al.* 2006; Harrisson *et al.* 2014). In metapopulations, population connectivity ensures that genetic diversity is maintained within habitat patches, through facilitation of gene flow that ameliorates the effects of genetic drift, a common cause of genetic diversity loss in small populations (Frankham 2005). Effective management of species in fragmented environments requires an understanding of population connectivity and its implications for contemporary population persistence and future adaptation to environmental change (Allendorf & Luikart 2007; Hughes *et al.* 2013; Pavlova *et al.* 2017).

Population connectivity is driven by two key factors: structural and functional connectivity (Hughes *et al.* 2013). Structural connectivity refers to influential environmental variables, such as distance between patches and presence of barriers (Manel *et al.* 2003; Fullerton *et al.* 2010). Functional connectivity refers to species' responses, including life history strategies, and dispersal ability and proclivity (Hughes *et al.* 2009; Öckinger *et al.* 2010). In the same environment, population connectivity often differs strongly among species due to differences in functional connectivity (Murphy *et al.* 2015). For example, species of volant aquatic insects exhibited much greater genetic connectivity across a desert landscape than did flightless ones (Phillipsen *et al.* 2015).

Desert freshwaters, especially those with ephemeral flows, are highly-fragmented environments, providing an ideal opportunity to study the impacts of functional connectivity on population connectivity (Kingsford 2006; Murphy *et al.* 2015). Many aquatic taxa, such as fish, exist as metapopulations in desert freshwaters, with subpopulations inhabiting isolated waterbodies that are temporarily reconnected during flow events (Datry *et al.* 2016). Facilitating persistence in desert freshwaters, aquatic species lie along a spectrum of persistence strategies from resistance to resilience (Bogan *et al.* 2017). A resistance strategy allows species to maintain subpopulations through tolerance of environmental extremes (e.g. drought, high temperature and salinity), whereas a resilience strategy facilitates recolonisation from distant subpopulations after flows resume (Lake 2000; Nimmo *et al.* 2015). Because they incorporate diverse aspects of a species' ecology, including dispersal ability and environmental tolerance, these strategies are a useful basis on which to frame explorations of the impacts of differences in functional connectivity on population connectivity.

Different persistence strategies are expected to lead to differences in population connectivity within a desert freshwater system for several reasons. Extended drought periods will often restrict resilient species to a small number of waterbodies, while resistant species often persist in a larger number of waterholes across a wider part of the system due to greater environmental tolerances (e.g. McNeil & Schmarr 2009). When flows resume, resilient strategists may disperse large distances and recolonise many waterbodies rapidly, whereas resistant species recolonise fewer (and closer) sites more slowly. Consequently, resistance-strategists are expected to show strong differentiation among subpopulations (i.e. genetic structure) and low gene flow, and resilience-strategists the converse pattern (Table 1). These patterns will be dependent on spatial scale; for example, all taxa should show greater structure among than within rivers. These patterns of genetic structure and gene flow have been summarised as 'connectivity models' by Murphy *et al.* (2015), as a proxy for genetic connectivity (Table 1).

Table 1. Predicted genetic patterns, within and among river systems in a single hydrological basin, of desert fishes with different persistence strategies, including their expected levels of genetic structure, gene flow, connectivity model (i.e. model of spatial population genetic structure), and the predicted relationship between genetic and geographic (river-channel) distances (adapted from Hutchison & Templeton 1999; Phillipsen *et al*. 2015).

	Persistence Strategies	
	Resistance	**Resilience**
Within-River Structure		
Level of Genetic Structure	Moderate	Low
Level of Gene Flow	Moderate	High
Connectivity Model	Stream Hierarchy Model	Panmixia
Spatial Structure		
Among-River Structure		
Level of Genetic Structure	High	Moderate
Level of Gene Flow	Low	Moderate
Connectivity Model	Death Valley Model	Stream Hierarchy Model
Spatial Structure		

Here, the highly fragmented river systems of the Lake Eyre Basin (LEB) in arid central Australia (Fig. 1) and a suite of diverse, co-distributed desert fish taxa of known persistence strategies are utilised to test the expectations outlined above. The LEB is an extreme and highly variable desert environment with no permanently flowing rivers, in which aquatic habitats are limited to a variable number of isolated waterholes during droughts (Kotwicki 1986; McMahon *et al.* 2005). During droughts, the environmental conditions within waterholes decline, and most will dry completely during extended droughts (Unmack 2001b; see Chapter Three for further details). However, the LEB is home to a diverse range of fishes (Wager & Unmack 2000; see Chapter Four for further details). Five of these are studied here, including two resilience-strategists, two resistance-strategists, and one intermediate strategist (Table 2). The species' persistence strategies were identified based on field

observations of responses of fishes to drought and subsequent flood events within the Neales River in the LEB (McNeil & Schmarr 2009; McNeil *et al.* 2011b). Researchers classified resistance-strategists as those that maintained populations in a number of waterholes during drought periods and recolonised slowly and locally following flood events. In contrast, resilience-strategists were defined as those that maintained one or very few populations during drought, but rapidly recolonised many sites once flows returned (for further information see Chapter Four). Post-drought recolonisation by LEB fishes have since been observed in the Georgina River, with similar responses reported for all species (Kerezsy *et al.* 2013).

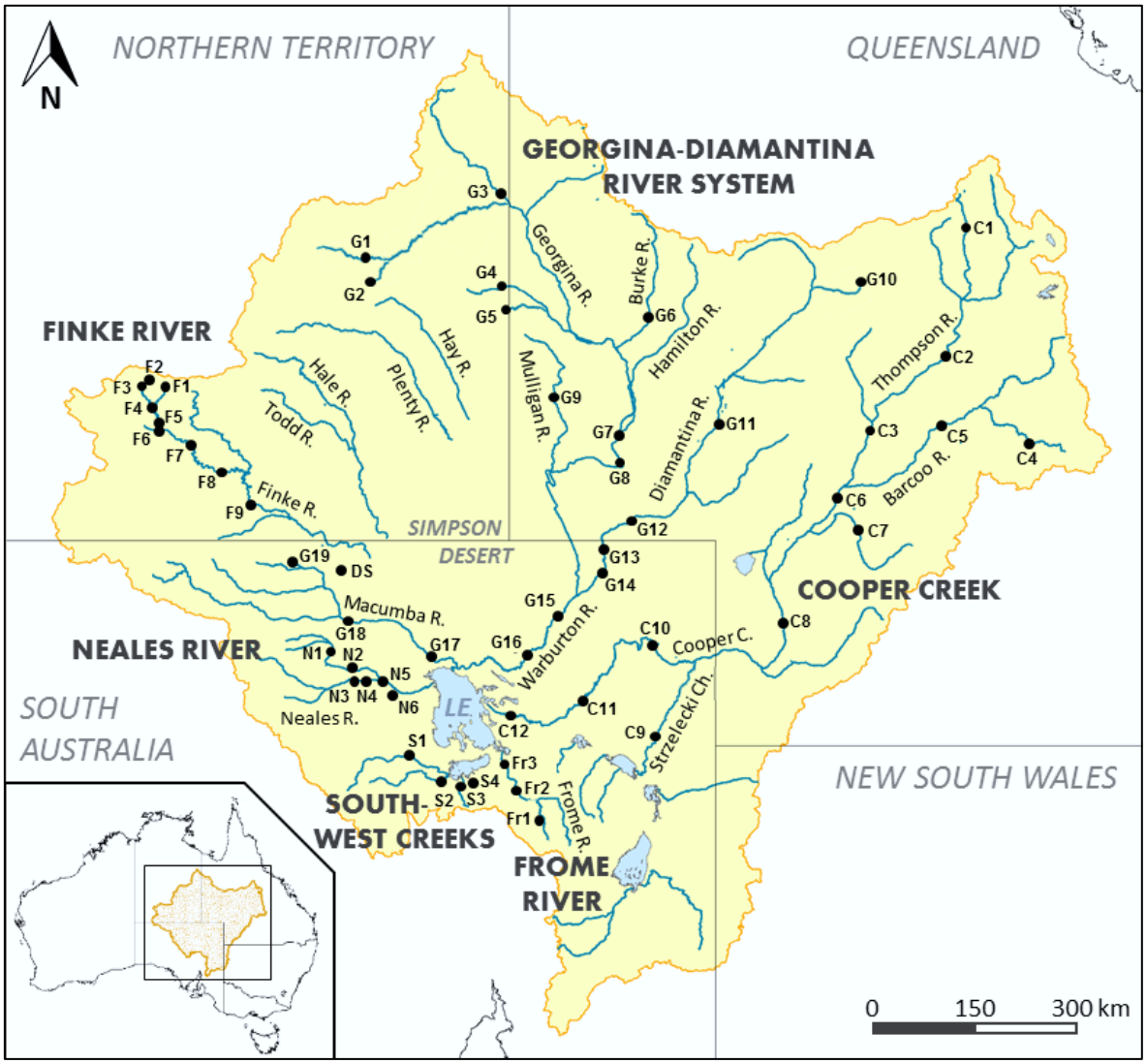


Figure 1. Sampling locations (dots) across six major river systems of the Lake Eyre Basin, including the Finke River (sites labelled "F"), Georgina-Diamantina River System (G), Cooper Creek (C), Frome River (Fr), South-West Creeks (S), and Neales River (N). For a detailed list of sites and samples, see Appendix 2.1.

Table 2. Study taxa, including two species-pairs analysed together (two *Chlamydogobius,* two *Craterocephalus*), and their *a priori* persistence strategies (following McNeil *et al.* 2011b), relative dispersal ability, relative tolerances of environmental extremes, and mean adult standard length (SL; mm, excluding tail) in the Lake Eyre Basin (Crowley & Ivantsoff 1990; Larson 1995; Wager & Unmack 2000; for further details see Chapter Four; Pusey *et al.* 2004).

Family	Taxon	Strategy	Dispersal	Tolerance	SL
Gobiidae	Finke Goby *Chlamydogobius japalpa*	Resistance	Weak	Strong	50-60
	Desert Goby *Chlamydogobius eremius*	Resistance	Weak	Strong	50-60
Atherinidae	Finke Hardyhead *Craterocephalus centralis*	Resistance	Moderate	Strong	50-65
	Lake Eyre Hardyhead *Craterocephalus eyresii*	Resistance	Moderate	Strong	50-100
Terapontidae	Barred Grunter *Amniataba percoides*	Intermediate	Moderate	Weak	100-200
	Spangled Perch *Leiopotherapon unicolor*	Resilience	Strong	Moderate	150-300
Clupeidae	Bony Herring *Nematalosa erebi*	Resilience	Strong	Weak	150-300

The population connectivity consequences of different persistence strategies for desert fishes have not previously been resolved. Understanding these consequences is useful for predicting the future effects of climate change and other anthropogenic impacts that are expected to further reduce structural connectivity in desert freshwaters globally. The aim of this study was to determine whether different persistence strategies influence the population connectivity of LEB fishes. It was hypothesised that resistant fishes will exhibit lower population connectivity than will resilient taxa (summarised in Table 1). To test this hypothesis, a range of population genetic analyses were performed on a suite of five diverse fish taxa sampled across the LEB. This study addresses how species' ecology drives population connectivity, with applications for understanding and managing the contemporary and future persistence of fishes and other aquatic species in desert freshwaters.

Methods

Study Taxa and Sampling

Seven fish species were included in this study (Table 2). Three are widespread across the Lake Eyre Basin (LEB) in central Australia, including the bony herring, *Nematalosa erebi*; spangled perch, *Leiopotherapon unicolor*; and barred grunter *Amniataba percoides*. Two others are found throughout LEB rivers that are (rarely) hydrologically connected via Lake Eyre, and have sister species restricted to the hydrologically isolated Finke River. These are the desert goby, *Chlamydogobius eremius* and Lake Eyre hardyhead, *Craterocephalus eyresii*, and Finke goby, *Chlamydogobius japalpa* and the Finke hardyhead, *Craterocephalus centralis*. For the purposes of genomic comparisons here, the closely-related pair of goby species were analysed together, as were the two hardyhead species. Hereafter, these combined taxa are referred to as 'goby' and 'hardyhead', respectively. Chapter Four presents further details on the taxonomy, biology and ecology of these species.

The five taxa were sampled in waterholes of the major river systems of the LEB including the Finke, Neales, and Frome rivers, the Georgina-Diamantina River System, Cooper Creek, and four smaller watercourses that drain into Lake Eyre South, termed 'South-West Creeks' (Fig. 1, Table 2, for full details see Appendix 2.1). Samples from springs were not included, except where adjacent to a river system. A single exception was samples of perch obtained from the isolated Dalhousie Springs complex, included for comparative purposes. An even sampling strategy across species was not possible due to the heterogeneous distribution of species at local scales and the absence of some species across part or all of some river systems (more information on species distributions is provided in Chapter Four).

Samples were obtained from existing museum or research collections, and during fieldtrips undertaken from September 2013 to October 2014. Fieldtrip samples were collected under ethics permissions obtained from Monash University Animal Ethics Committee (BSCI_2013_28), and by permission of the relevant wildlife authorities, including the South Australian Department of Environment, Water and Natural Resources (No. Q26166-2), Primary Industries and Regions South Australia (No. ME9902683), Northern Territory Department of Primary Industry and Fisheries (No. 2014-2015/S17/3341), and the Parks and Wildlife Commission of the Northern Territory (No. 51620). Fish were caught with dip or

seine nets, or mesh traps. As only small volumes of tissue were required, fin clips were taken from most specimens and the fish released. For very small fish, whole specimen samples were taken. Samples were stored in in 95% ethanol, and frozen as soon as possible. Additional samples were received from the collections of the South Australian Museum, Museum and Art Gallery of the Northern Territory, and Queensland Department of Natural Resources and Mines. In total, between 83 and 229 samples were obtained for each taxon (Table 3).

Table 3. Sample sizes of each taxon collected in the river systems of the Lake Eyre Basin. In addition, five spangled perch were sampled in the isolated Dalhousie Springs complex, considered separately in this study. For a detailed list of sample sites, see Appendix 3.1.

Taxon	Finke River	Neales River	South-West	Frome River	Cooper Creek	Georgina-Diamantina	Total *n*
Goby	67	43	32	9	-	24	175
Hardyhead	70	24	34	3	1	10	142
Grunter	52	18	-	-	-	13	83
Perch	57	20	1	1	29	43 (5)	156
Herring	35	17	-	9	70	98	229

Molecular Data Collection

As DNA sequencing costs continue to decline, the cost of enrichment-based targeted sequencing approaches will increasingly be in the library preparation and enrichment steps. One way to reduce these costs is to pool samples prior to library preparation. However, as library fragments are not yet indexed, sequence reads would need to be sorted without indexes prior to downstream processing. The degree to which this is a problem is a function of the level of sequence divergence among the samples being pooled. If samples are pooled before library preparation in such a way that they can be distinguished based on their target sequences, then sample preparation costs can be reduced dramatically and processing rate increased, without effect on data quality. We term this approach to sample preparation MetaPrep, which will be described in more detail in an upcoming work (Lemmon *et al.* in prep.). Such an approach has not been used before, and this work is part of an initial trial of the novel MetaPrep technique, which included one bird (data utilised in Morales *et al.* 2017a, 2017b), seven invertebrate taxa (data utilised in Razeng 2018) and one other fish taxon (data yet to be published), in addition to the five fish taxa studied within this project.

Enrichment probe design

A high-throughput targeted sequence capture approach was used to obtain sequence data for up to 1,000 anonymous nuclear loci per taxon. To identify candidate loci, low-coverage genome sequencing (paired-end 150 bp Illumina, with C-bot clustering yielding ~3.25 Gb per taxon, ~2–8X coverage) was performed on one representative individual of each taxon. Sequencing was performed at the Translational Science Laboratory at Florida State University. Quality-filtered reads were merged following Rokyta *et al.* (2012). From the pool of merged reads, 2,000 candidate targets per taxon were selected based on GC content (40–55%, to increase enrichment uniformity) and length (180–240 bp, to ensure locus length uniformity). To assess approximate copy number in the genome of candidate targets and check for cross-taxa mapping, the full set of merged reads were shallow-mapped to the 10,000 candidate regions over the five taxa, using a preliminary 25-kmer exact match and a final 72 of 120 consecutive base pair match requirement (Lemmon *et al*. in prep). Within-taxon assembly profiles were used to remove coverage-outlier candidates, by requiring five or six reads mapped and at least 96% average identity across mapped reads. Cross-taxa assembly profiles were used to remove loci for which cross-taxa mapping occurred. These filters retained 1,018–1,473 loci per taxon, of which 1,000 were randomly selected to serve as targets for hybrid enrichment. Probes (120 bp each) were tiled uniformly at 2x density (three probes per locus) to form the probe set.

Library preparation

Total genomic DNA was extracted from each sample using DNeasy tissue extraction kits (Qiagen, Venlo, the Netherlands). All downstream handling, up to the bioinformatics stage (see below), was conducted at the Center for Anchored Phylogenomics at Florida State University. Following quantification of extracted DNA using the *Qubit*® fluorometer, one sample from each taxon were pooled in equal concentrations (Lemmon *et al*. in prep.). Each MetaPrep pool (MetaPool) contained only one individual from each taxon to enable identification of sequenced alleles for each individual. An indexed library was then prepared from each MetaPool following Lemmon *et al.* (2012). Briefly, MetaPooled DNA was sonicated using a Covaris E220 focused-ultrasonicator to a fragment size of ~300–800 bp. Subsequent library preparation and indexing was performed on a liquid-handling robot (Beckman-Coulter Biomek FXp) following Meyer and Kircher (2010), with the modification that samples were size-selected after blunt-end repair using SPRI select beads

(Beckman-Coulter Inc.). Each MetaPool was given a unique 8 bp index, with indexes differing by a minimum of two base pairs.

Enrichment and sequencing

Indexed MetaPools were pooled at equal quantities for enrichment (24 MetaPools per enrichment pool) using an XT Agilent Sure Select enrichment kit containing probes for all taxa. Libraries were sequenced on an Illumina 2500 lane with paired-end 150 bp reads and 8 bp indexing read.

Read assembly

Following sequencing, default parameters in Illumina's CASAVA software were used to filter out low-quality clusters and reads. Reads were de-multiplexed by MetaPrep index, with no mismatches tolerated. Sequencing errors were corrected when merging overlapping regions of paired reads, using quality scores set to Bayesian posterior estimates (Rokyta *et al.* 2012). Reads were then de-MetaPrepped (i.e. sorted into collections of sequences belonging to the single individual of each taxon within a MetaPool). Briefly, reference sequences used in probe design were extended into flanking regions using the raw genomic reads. Reads from each MetaPrep pool were shallow-mapped (as above) to the full set of 5,000 extended reference target sequences. Reads with a unique mapping location were retained, sorted by taxon, and assembled using the extended references (Hamilton *et al.* 2016; Prum *et al.* 2015). Only consensus sequences with at least 20x reads mapped were utilised for downstream analysis to ensure adequate depth of sequencing for accurate calling of variation. After assembly, heterozygous sites were identified using a binomial model that determines the probability that site variation resulted from 1% sequencing error, or heterozygosity. If sequencing error was supported, it was corrected by selecting the most globally common base call for the consensus sequence. If heterozygosity was supported, the appropriate ambiguity base was called for the consensus sequence. A minimum of 10x coverage was required for calling bases for which reads disagreed on the base call; ambiguities were used to represent the uncertainty when less than 10x coverage was obtained. Allele phasing for each locus was determined statistically from the assembled reads, by drawing a posterior distribution for each individual, following Pyron *et al.* (2016). This method generates alleles with ambiguities only at those positions that cannot be phased with a $\geqslant$95% posterior probability confidence.

Bioinformatics

Two datasets were constructed for each taxon. A phased sequence dataset consisted of the aligned phased sequence data for each locus in each individual. This included polymorphic and monomorphic loci. A genotype dataset was also generated from the phased sequence dataset, with monomorphic loci removed. Sequences were then reduced to only polymorphic sites by calling single nucleotide polymorphisms (SNPs) from alignments using the R package *adegenet* v2.01 (Jombart & Ahmed 2011; R Core Team 2014). Custom R scripts (see Appendix 2.2) were then used to filter out SNPs based on missing data (>20% for SNPs, >50% for individuals), heterozygosity (>0.8) and minor allele frequency (MAF <0.02). For each locus, a custom R script (Appendix 2.2) was used to combine the phased SNPs as alleles to create the genotype dataset.

For analyses that require loci free from signals of natural selection, the phased sequence and genotype datasets were reduced to contain only putatively neutral loci. Putatively non-neutral loci were identified from genotypes using BayeScan v2.1 (Foll & Gaggiotti 2008), which employs a Bayesian likelihood method based on a logistic regression model that separates population-specific effects of demography from locus-specific effects of selection. F_{ST} coefficients (Beaumont & Balding 2004) are estimated and decomposed into population-specific (β) and locus-specific (α) components, with α values that differ significantly from zero identifying a departure from neutrality. Following 20 preliminary runs of 5,000 iterations after a burn-in of 50,000 iterations, we used 100,000 iterations (sample size of 5,000 and thinning interval of 20) and a threshold false discovery rate (FDR) of 0.1 to identify loci potentially under selection. Loci identified as putatively non-neutral were removed to generate putatively neutral datasets (in addition to complete datasets) for relevant analyses.

Estimation of Genetic Diversity

To examine patterns of neutral genetic diversity within each taxon across the LEB, summary genetic statistics were calculated using the putatively neutral genotype dataset. At the river scale, diversity metrics included the proportion of monomorphic loci and the proportion of the total allelic diversity present within each river system, calculated using the genotype dataset. The mean number of private alleles per locus within each river system was also calculated, using GenAlEx 6.502 (Peakall & Smouse 2006). Four additional metrics were

calculated for each waterhole, and averaged to give a mean waterhole-scale diversity value for each river. The sample-size adjusted allelic richness (AR), and observed and expected heterozygosities (H_O, H_E) of each waterhole were determined using the R package *DiveRsity* (Keenan *et al.* 2013). Nucleotide diversity (π) was calculated from the sequence dataset, using the R package *PopGenome* (Pfeifer *et al.* 2014).

Characterisation of Population Structure

To examine the population subdivision of each taxon across the LEB, a variety of approaches were conducted. For initial explorations and visualisation of structure, two dissimilar yet complementary clustering algorithms were utilised: STRUCTURE (Pritchard *et al.* 2000) and discriminant analysis of principal components (DAPC; Jombart *et al.* 2010). STRUCTURE is commonly used for exploration of population structure and is generally effective at finding biologically realistic subdivisions (Meirmans 2015). However, its reliance on explicit population models requires assumptions, including that populations are in Hardy-Weinberg equilibrium, which may not hold for LEB fishes (Pritchard *et al.* 2000). Unlike STRUCTURE, DAPC makes no assumptions about population genetic models, and may be more effective at identification of genetic clines and hierarchical structure (Jombart *et al.* 2010). Both approaches were implemented here, as different methods may be informative of different aspects of population structure (Waples & Gaggiotti 2006).

The Bayesian, individual-based non-spatial clustering algorithm STRUCTURE v2.3 (Pritchard *et al.* 2000; Falush *et al.* 2003) was conducted at two scales for each taxon. Initial runs were conducted across the LEB using all samples, followed by runs for each river separately to elucidate within-river structure. STRUCTURE was run using the admixture model with correlated allele frequencies, and the number of genetic clusters (K) set from 1 to 10. Twenty replicates of 1,000,000 Markov Chain Monte Carlo runs following a burn-in period of 500,000 repetitions were performed for each value of K. Results of all runs were visualised using CLUMPAK (Kopelman *et al.* 2015) and summarised using STRUCTURE HARVESTER v0.6.94 (Earl & von Holdt 2011). The summary statistic ΔK (Evanno *et al.* 2005), as well as the biologically informative structure observed (Meirmans 2015), were considered when interpreting the most likely number of clusters.

The DAPC was conducted on all samples across the LEB, again utilising the genotype dataset, using *adegenet*. DAPC uses data transformed by a principal components analysis (PCA) to define synthetic variables in which genetic variation is maximised between, and minimised within, clusters of individuals. The K-means clustering algorithm, which identifies groups that maximise differences between groups, was run sequentially for each K value, and the best-supported K was determined using the Bayesian Information Criterion.

Quantification of Population Structure

To quantify the population structure of each fish taxon across the LEB, several distinct metrics were calculated. To describe hierarchical genetic structuring within and among the rivers of the LEB for each taxon, we utilised an analysis of molecular variance (AMOVA; Excoffier *et al.* 1992). Analyses were based on genetic distances and conducted using ARLEQUIN (Excoffier & Lischer 2010). Significance levels were assessed using 10,000 permutations. An AMOVA was conducted for all sites for each taxon. In addition, because of the uneven species' distributions and sampling, comparable AMOVAs were calculated using only samples from rivers in which all taxa were sampled – the Finke, Neales and Georgina-Diamantina rivers. A further AMOVA was calculated using the same approach but excluding the Finke River, to determine the relative impact of this hydrologically-disconnected river on observed structure.

Second, the allele frequency differentiation between each sampled river system, and between each sampled waterhole, was estimated with Nei's (1973) pairwise F_{ST} estimates. These estimates were made using the R package *Hierfstat* v2.01 (Goudet 2005). To determine if population differentiation was significant, a null-distribution of F_{ST} values was obtained by computing 1,000 permutated F_{ST} matrices with individuals randomly distributed between groups. To calculate *p*-values, the proportion of null-distribution estimates that were higher than observed F_{ST} values were counted. Global F_{ST} estimates were also made using *Hierfstat*, using Weir & Cockerham's (1984) unbiased measure.

The fact that F_{ST} is dependent on the observed level of diversity presents challenges for comparing species that differ in their effective population sizes, or when different loci (with different mutation rates) are utilised (Hedrick 2005). Accordingly, G''_{ST}, an F_{ST} analogue standardised by the maximum value attainable given observed within-population diversity

(Meirmans & Hedrick 2011), was calculated. G''_{ST} avoids biases reported in similar metrics (G_{ST}, G'_{ST}) when the number of populations is small (Meirmans & Hedrick 2011). Pairwise G''_{ST} values and associated 95% confidence intervals were calculated between each river, and between each waterhole, using *DiveRsity*. Values were considered significant at the 0.05 level where confidence intervals excluded zero. Global G''_{ST} values were also calculated.

Finally, to investigate the relationship between genetic and geographic distances, isolation by distance (IBD) was tested across rivers in which all taxa were sampled. Two separate analyses were performed for each taxon, one for the hydrologically-connected Neales and Georgina-Diamantina rivers, and one for the isolated Finke River. Mean pairwise genetic distances between individuals was calculated with the unbiased estimator Rousset's $\hat{a}$ statistic (Rousset 2000; Watts *et al.* 2007), using the program GenePop v4.6 (Rousset 2008). Geographic distances between individuals were determined by calculation of river channel distances between sampled waterholes, using the Network Analyst tool in ArcGIS v10.3 (ESRI). The correlation between genetic and geographic distances was tested using Mantel tests (1,000 permutations), with confidence intervals around the slope of the regression estimated by bootstrapping over loci in GenePop v4.6 (Rousset 2008). The minimum geographic distance for regression analysis was 0.1 km.

Determination of Patterns of Recent Gene Flow

To determine levels and directions of recent gene flow among populations of fishes among and within the rivers of the LEB, two complementary assignment test analyses were conducted. First, potential first-generation migrants between rivers were identified using assignment tests implemented in the program GENECLASS2 (Piry *et al.* 2004). Analyses were conducted among all LEB river systems. The program was run with the Paetkau *et al.* (2004) frequencies-based computation criteria and the authors' recommended settings for missing allele frequencies. Assignment probability was based on the likelihood of the individual genotype in its capture population compared to the highest likelihood among all population samples (L = L_home/L_max), and was assessed through Monte Carlo simulations of 10,000 individuals with the algorithm of Paetkau *et al.* (2004) and a rejection threshold of 0.05.

Second, recent migration rates between rivers, and between waterholes within rivers, were inferred using the Bayesian Monte Carlo Markov Chain (MCMC) approach implemented in the program BayesAss (Wilson & Rannala 2003). BayesAss defines the migration rate (*m*) into a population per generation as the proportion of individuals in the population derived from other populations within the last three generations. Analyses were conducted among rivers, and within each river individually (with samples from the focal system separated by waterhole, and other samples grouped by river system). A random subset of 400 loci was used for each taxon, due to computational limits of BayesAss. Five independent runs were conducted for each analysis, with 55,000,000 iterations performed following a burn-in of 5,000,000 iterations, and a sampling interval of 100. Adjustments to delta values for allele frequency, mutation rate and inbreeding were made to optimise the acceptance rates. Convergence was checked by examining tracer plots in Tracer v1.6 (Rambaut & Drummond 2007). Mean migration rates were calculated for each analysis by averaging across the inferred (posterior mean) migration rates of the five replicate runs, with 95% credible intervals calculated using this mean ± 1.96 * mean standard deviation.

Results

MetaPrep Dataset Characteristics

The phased sequence datasets generated from the MetaPrep data were very similar in size among taxa, and contained a mean of 810 loci per taxon, with a mean sequence length of 597 base pairs (see Appendix 2.2 Table 1). The longest sequence generated was 1141 base pairs (grunter), while the shortest retained sequence was 152 base pairs (hardyhead). The mean number of monomorphic loci was 142 per taxon, with a range of 64 monomorphic loci (goby) to 243 monomorphic loci (spangled perch). The genotype dataset had a mean of 668 loci per taxon, with a mean of 5.16 genotypes per locus. The number of genotypes per locus ranged from 4.35–4.69 for goby, hardyhead and barred grunter, but were substantially higher for spangled perch and bony herring (6.10–6.15; for full details for each taxon, see Appendix 2.2 Table 1). The selection detection analyses identified from 2 to 20 loci per taxon as putatively non-neutral, with herring having the most putatively non-neutral loci (SI Appendix 2.2 Table 2).

Genetic Diversity of Fish Populations of the Lake Eyre Basin

Resistant and resilient strategists exhibited similar patterns in genetic diversity metrics across the LEB. Populations of all five taxa within the Finke River generally had lower diversity than those in other river systems (Table 4). For example, only 16% of loci were polymorphic in the resistant goby population in the Finke, compared with 81% in the Neales River. Waterhole-scale populations of resilient spangled perch in the Finke had expected heterozygosity levels at least 25% lower than those in other river systems. There were few exceptions to this pattern of lower diversity in the Finke, although the proportion of private alleles showed a different pattern, which appeared correlated with sample size (more private alleles were identified when sample size was larger). All taxa showed less substantial differences in genetic diversity levels among the other LEB rivers, with no consistent patterns among these river systems.

While there were differences in levels of genetic diversity among rivers, the differences among waterholes within rivers were minimal. For example, the resilient bony herring in Cooper Creek had observed heterozygosities that ranged from 0.240 to 0.311 across ten waterholes, while the resistant hardyhead exhibited allelic richness of 1.35–1.48 across six waterholes in the Finke River (see Appendix 2.3 for within-river genetic diversity statistics for each taxon). This pattern of similar levels of genetic diversity in waterholes within any given river was consistent across all taxa. No consistent trends in longitudinal diversity (i.e. differences between upstream and downstream waterholes) were apparent for any taxa or river systems.

Table 4. Genetic diversity statistics for the five fish taxa sampled in the river systems of the Lake Eyre Basin, including M: percentage of loci that are monomorphic within each river system; A: proportion of the total number of alleles found in that river system; PA: mean absolute number of private alleles per locus; AR: mean allelic richness (adjusted for sample size of 5); H_O: observed heterozygosity; H_E: expected heterozygosity; π: nucleotide diversity (x 10^{-4}). Sample size in each river system is indicated by *n*.

			River Scale			Waterhole Scale			
Taxa	**River**	***n***	**M**	**A**	**PA**	**AR**	**H_O**	**H_E**	**π**
Goby	Finke	67	84.14	32.13	0.171	1.12	0.027	0.032	0.064
	Neales	42	18.84	63.30	0.174	1.86	0.192	0.224	0.453
	South-West	32	20.82	66.05	1.497	2.42	0.343	0.373	0.889
	Frome	9	57.22	40.14	0.035	1.50	0.140	0.168	0.351
	Geo-Dia	24	42.63	46.27	0.050	1.61	0.124	0.166	0.322
Hardyhead	Finke	70	38.46	53.13	0.736	1.49	0.202	0.224	0.399
	Neales	24	11.94	70.61	0.146	1.84	0.331	0.350	0.687
	South-West	34	7.96	78.28	0.422	1.98	0.362	0.382	0.783
	Frome	3	35.81	48.71	0.028	1.65	0.328	0.284	0.705
	Geo-Dia	10	24.54	56.98	0.046	1.66	0.275	0.303	0.644
Grunter	Finke	52	32.18	57.15	0.642	1.41	0.234	0.258	0.606
	Neales	18	6.23	75.36	0.503	1.61	0.400	0.473	1.314
	Geo-Dia	13	4.33	71.29	0.434	1.70	0.416	0.483	1.421
Perch	Finke	57	22.53	54.31	0.485	2.32	0.290	0.327	0.900
	Neales	20	11.27	57.91	0.131	2.87	0.401	0.443	1.252
	Cooper	29	8.64	60.36	0.606	3.06	0.438	0.449	1.327
	Geo-Dia	48	3.06	72.05	0.667	3.47	0.448	0.515	1.522
Herring	Finke	35	33.79	42.61	0.245	1.95	0.253	0.274	0.959
	Neales	17	4.58	60.56	0.138	2.71	0.396	0.417	1.419
	Frome	9	10.55	51.40	0.026	2.56	0.389	0.389	1.415
	Cooper	70	7.03	66.24	0.295	2.32	0.292	0.315	1.090
	Geo-Dia	98	0.61	88.45	1.147	3.11	0.426	0.462	1.579

Characterisation of Genetic Structure of Fish Populations of the Lake Eyre Basin

Strong genetic structure among some, but not all, river systems of the LEB was visualised for resistant and resilient fishes with discriminant analyses of principal components (DAPC; Fig. 2). The DAPCs gave most support to a structure with three clusters for all taxa except the resistant goby, for which six clusters was most likely. In all taxa, individuals sampled in the Finke River formed a distinct cluster. For species in which individuals from Cooper Creek or the South-West Creeks were sampled, these systems also formed distinct clusters (with the resistant goby showing two clusters in the latter river system; Fig. 2A). The remaining rivers, including the Neales, Frome and Georgina-Diamantina, which all flow into Lake Eyre North, were grouped into a single cluster for all taxa except grunter.

The among-rivers STRUCTURE analyses revealed similar patterns to DAPC (Figs. 3A–7A). The best-supported value of K, based on the Evanno *et al*. (2005) ad hoc ΔK method, was K=3 for herring and K=2 for the other taxa (data not shown). However, the most biologically informative number of clusters (shown in Figs. 3–7) was K=3 for all taxa except perch (K=4, with individuals from the remote Dalhousie Springs forming a distinct cluster for this species; Fig. 6A). For all taxa, individuals sampled in the Finke formed a distinct cluster, except for one goby individual from the Neales River (Fig. 3A) that was removed from further analyses (see Appendix 2.4 for further details about this individual). The resistant gobies showed the most distinct structuring, with no admixture between the Lake Eyre North (Neales, Frome and Georgina-Diamantina) and Lake Eyre South (South-West Creeks) clusters (see Fig. 1). In contrast, other taxa showed clusters that were present in all rivers, except the Finke.

The within-river STRUCTURE analyses found that while taxa exhibit varying degrees of structure at this scale, patterns were generally similar among hardyhead, grunter, perch and herring. The resistant gobies showed the greatest structure, with clusters in the upper and lower reaches of each river, or in separate watercourses in the case of the South-West Creeks (Fig. 3B–E). Within the Neales River, the STRUCTURE plot (Fig. 3C) closely matches the dendritic pattern of that river (Fig. 1). The other resistant taxon, hardyhead, the intermediate grunter, and the resilient perch and herring generally exhibited a lack of structure within rivers, although in some cases a small number of individuals clustered separately (Figs. 4–7B–E). Within the Georgina-Diamantina and Cooper systems, perch and herring show clusters separating upstream and downstream sites, with admixture present (Figs. 6–7D–E).

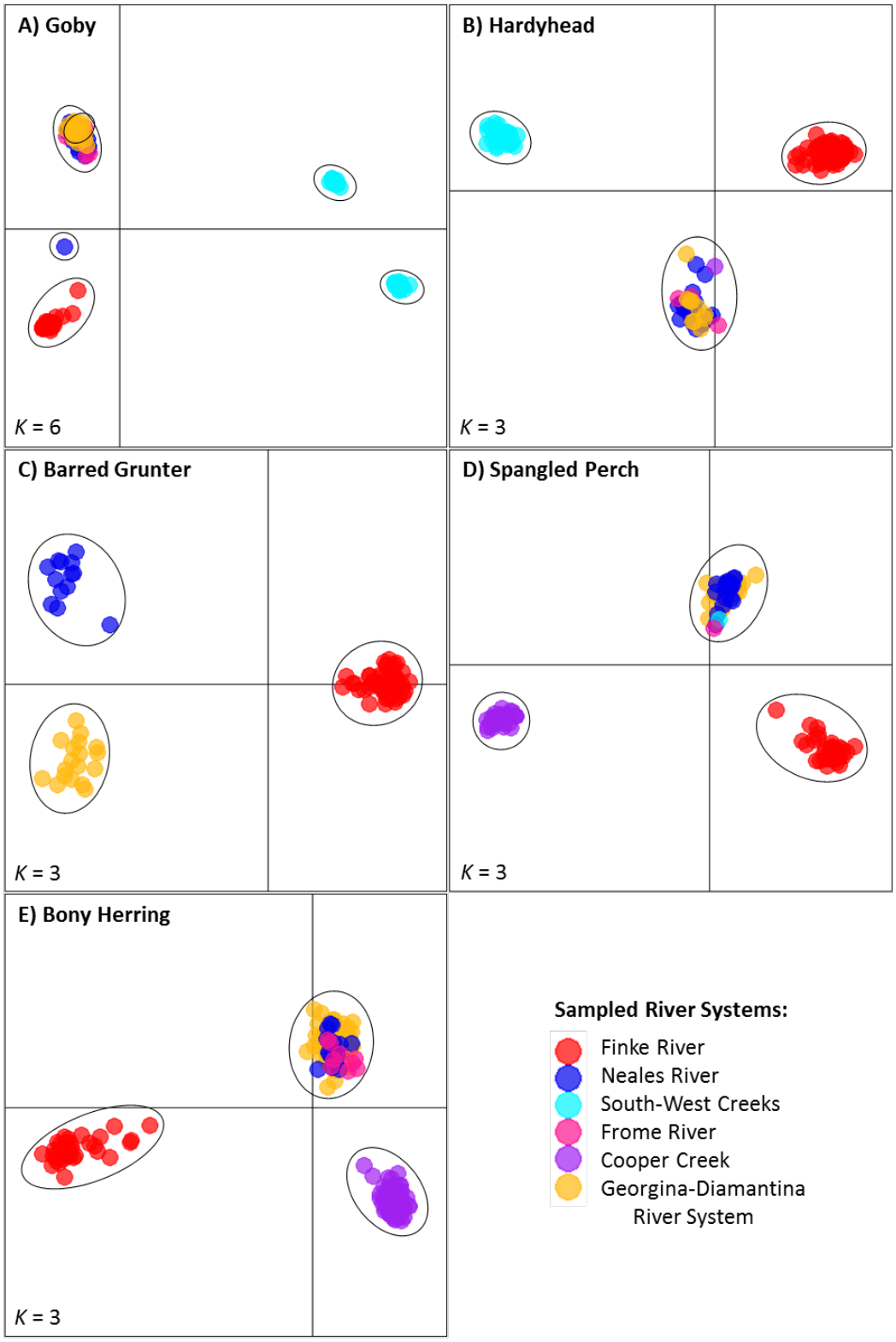


Figure 2. Discriminant Analysis of Principal Components (DAPC) based on genotype data for five fishes: A) goby; B) hardyhead; C) barred grunter; D) spangled perch; and E) bony herring; sampled across up to six river systems of the Lake Eyre Basin. Dots represent individuals, and are coloured based on sampling location (river system). The number of genetic clusters in each ordination plot is denoted by the K value; clusters are indicated with ellipses.

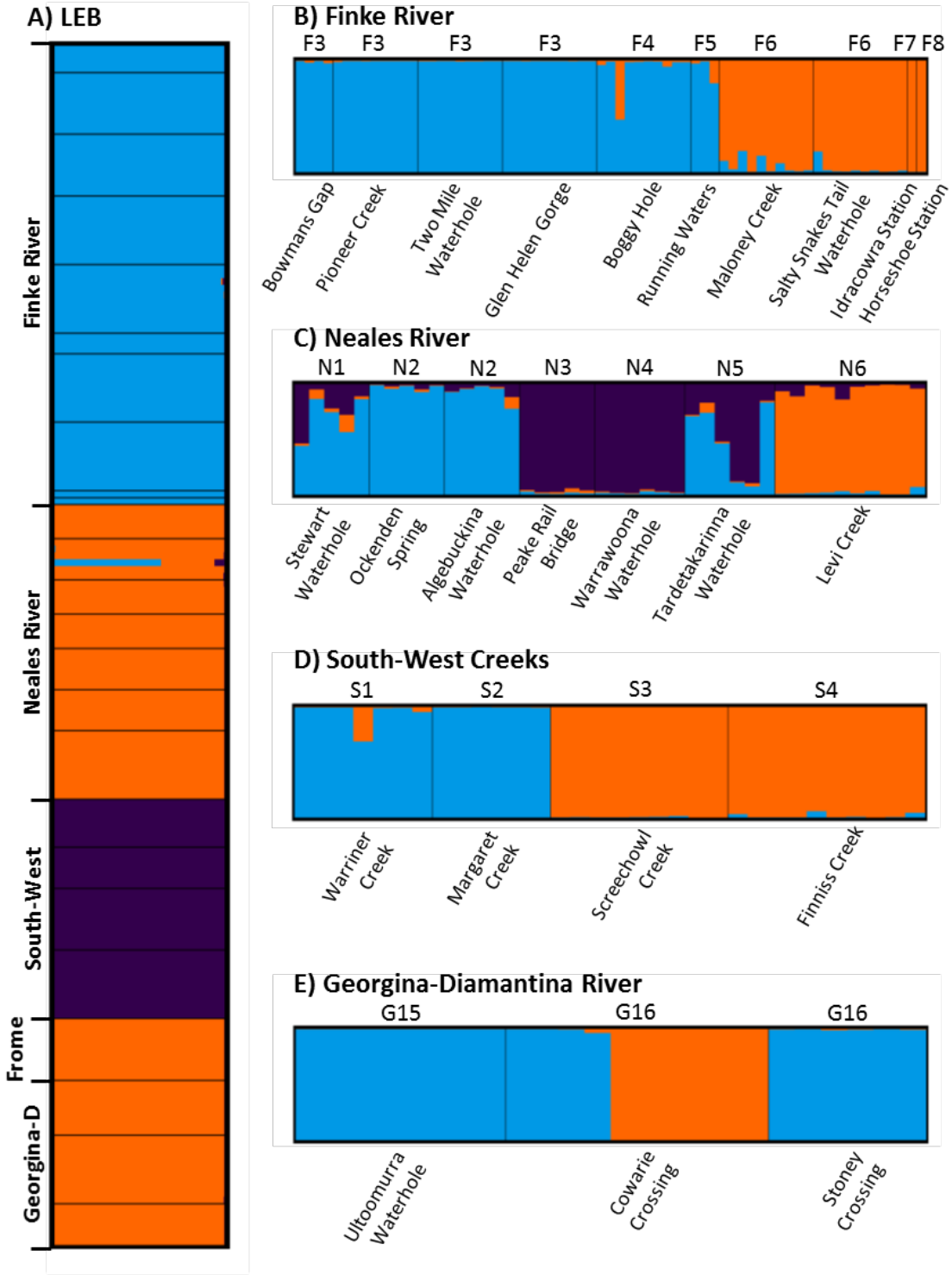


Figure 3. Population structure of desert goby *Chlamydogobius eremius* and Finke goby *Ch. japalpa* in A) the overall and B-E) individual river systems of the Lake Eyre Basin. The most likely number of genetic clusters, determined from analysis with the individual-based clustering algorithm STRUCTURE, is shown, with clusters represented by distinct colours. Within plots, each bar shows the proportion of an individual's genotype assigned to each cluster, and sampling sites (organised from upstream to downstream (left to right)) are separated by thin lines. For B-E), site codes are displayed above plots and mapped in Fig. 1.

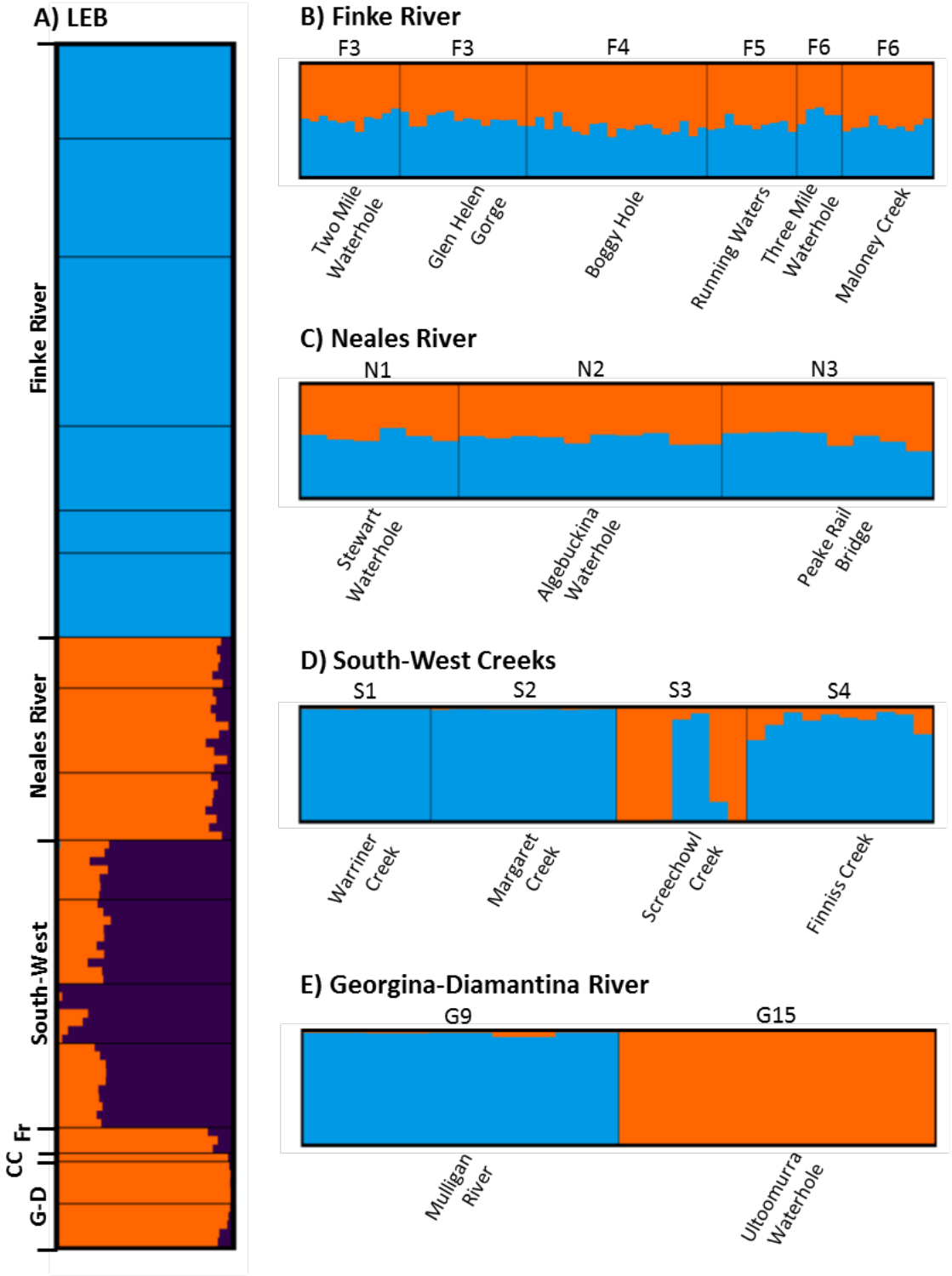


Figure 4. Population structure of Lake Eyre hardyhead *Craterocephalus eyresii* and Finke hardyhead *Cr. centralis* in A) the overall and B-E) individual river systems of the Lake Eyre Basin. The most likely number of genetic clusters, determined via STRUCTURE analysis, is shown, with clusters represented by distinct colours. Within plots, each bar shows the proportion of an individual's genotype assigned to each cluster, with sampling sites (organised from upstream to downstream (left to right)) separated by thin lines. In A) abbreviated names are as follows: Fr = Frome River, CC = Cooper Creek, G-D = Georgina-Diamantina River. For B-E), site codes are displayed above plots and mapped in Fig. 1.

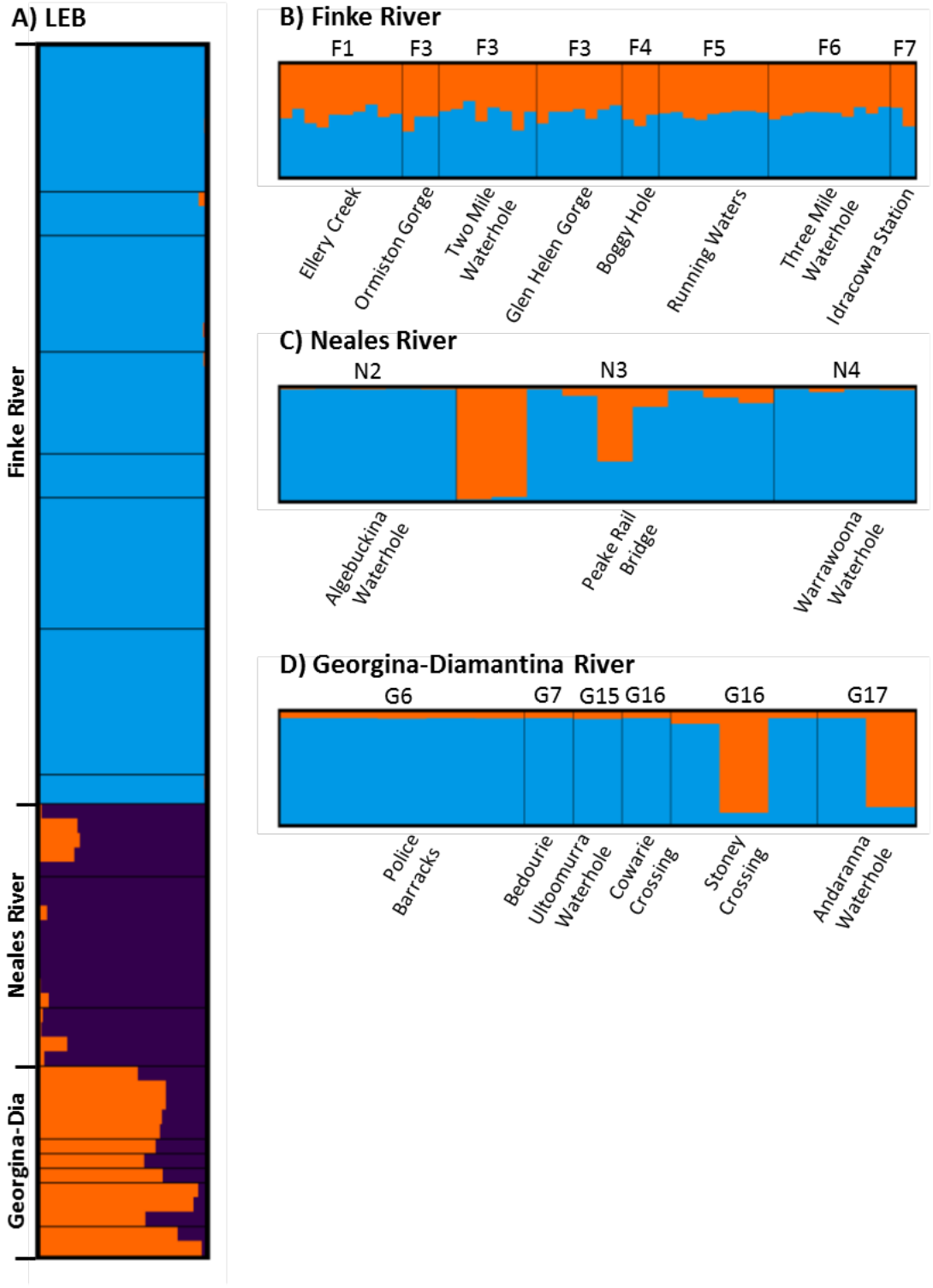


Figure 5. Population structure of barred grunter *Amniataba percoides* in A) the overall and B-E) individual river systems of the Lake Eyre Basin. The most likely number of genetic clusters, determined via STRUCTURE analysis, is shown, with clusters represented by distinct colours. Within plots, each bar shows the proportion of an individual's genotype assigned to each cluster, with sampling sites (organised from upstream to downstream (left to right)) separated by thin lines. For B-D), site codes are displayed above plots and mapped in Fig. 1.

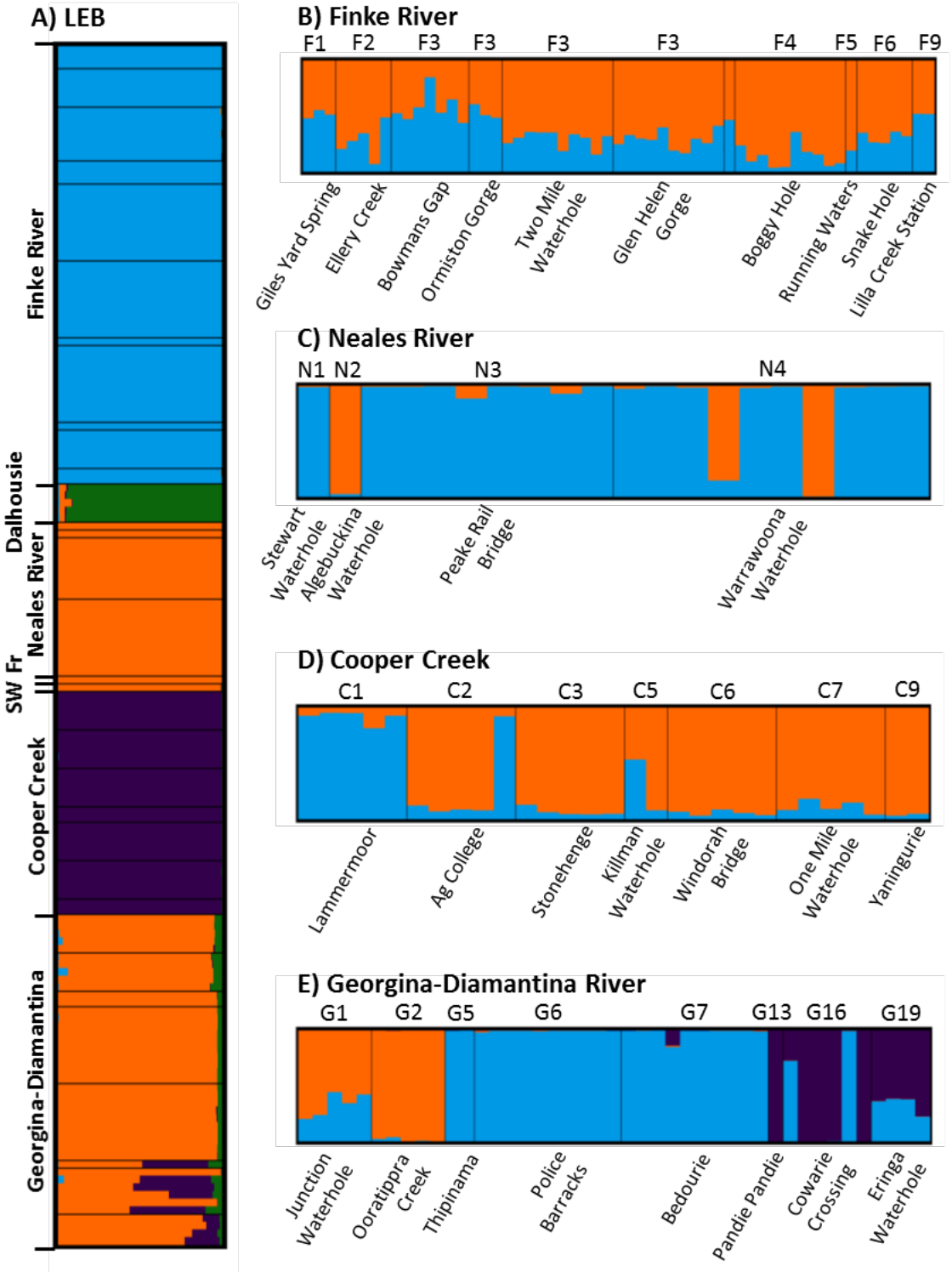


Figure 6. Population structure of spangled perch *Leiopotherapon unicolor* in A) the overall and B-E) individual river systems of the Lake Eyre Basin. The most likely number of genetic clusters, determined via STRUCTURE analysis, is shown, with clusters represented by distinct colours. Within plots, each bar shows the proportion of an individual's genotype assigned to each cluster, with sampling sites (organised from upstream to downstream (left to right)) separated by thin lines. In A) abbreviated names are as follows: Fr = Frome River, SW = South-West Creeks. In B-E) site codes are mapped in Fig. 1.

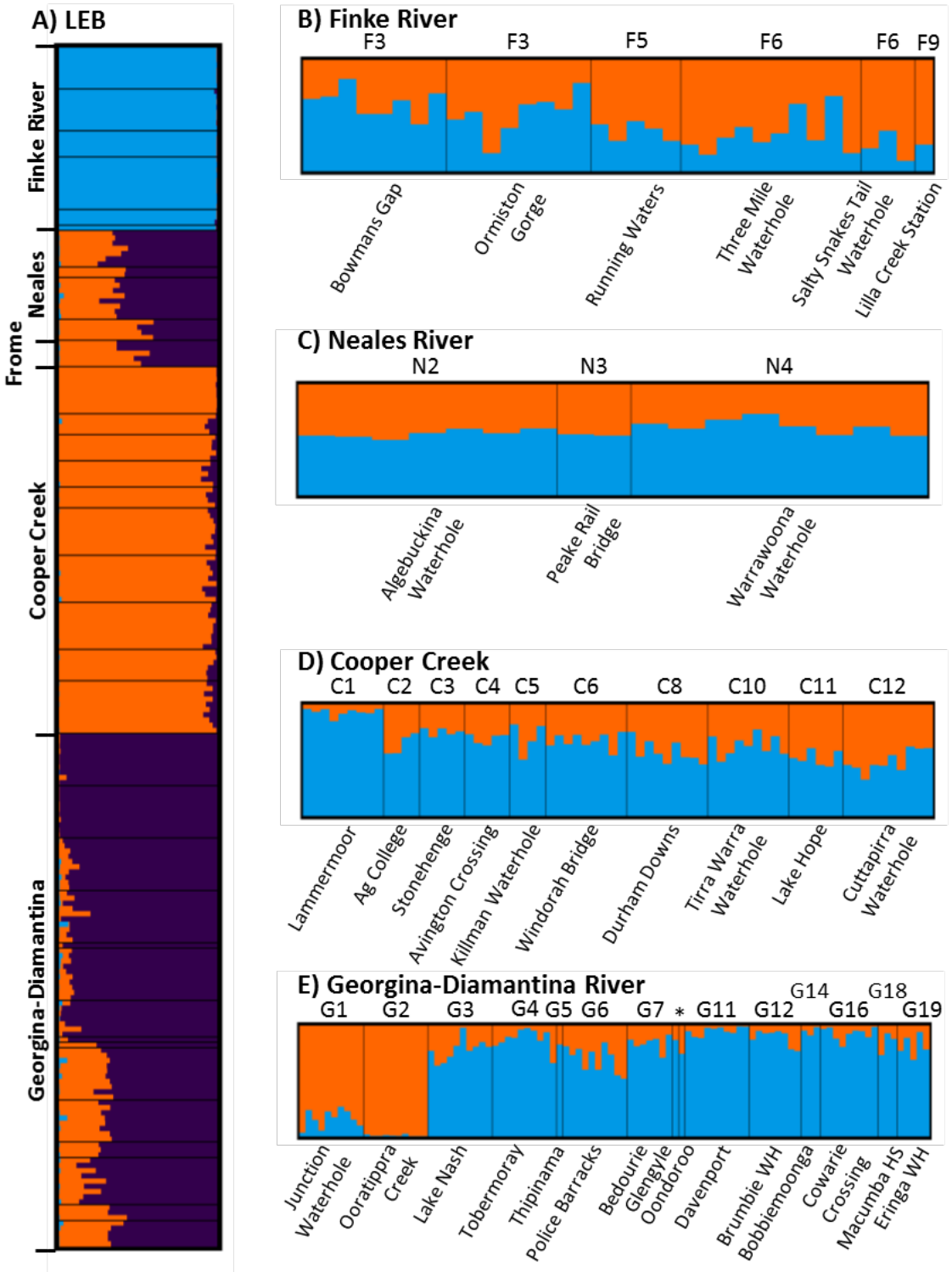


Figure 7. Population structure of bony herring *Nematalosa erebi* in A) the overall and B-E) individual river systems of the Lake Eyre Basin. The most likely number of genetic clusters, determined from analysis with the individual-based clustering algorithm STRUCTURE, is shown, with clusters represented by distinct colours. Within plots, each bar shows the proportion of an individual's genotype assigned to each cluster, and sampling sites (organised from upstream to downstream (left to right)) are separated by thin lines. In B-E) site codes are mapped in Fig. 1. In E) * denotes sites G8 and G10.

Quantification of Genetic Structure of Fish Populations of the Lake Eyre Basin

Analyses of molecular variance (AMOVA) show that among-river differences generally explained a larger proportion of variation for resistance-strategists than resilience-strategists, except when only the two hydrologically-connected rivers are considered. However, the resistant hardyhead showed values more similar to the intermediate grunter than the fellow resistant taxon goby (Table 5). For all taxa, a significant proportion of variation was explained by differences among rivers for all taxa, while differences within rivers explained very little (<5%). These results indicate that populations of all taxa have very little differentiation within river systems, largely following the structure visualisation analyses. When only the two hydrologically-connected rivers in which all taxa were sampled were considered, the proportion of variance explained by differences among rivers was greatly reduced for all taxa. While the resistant goby still showed strong differentiation among rivers, very little variation was explained by among-river differences for the remaining taxa.

Table 5. Results of analyses of molecular variance (AMOVA), including percentage of total variation, associated *F*-statistic and *P*-values from significance tests of the comparison of each hierarchical level, calculated for five fish taxa sampled across the Lake Eyre Basin. AMOVAs were performed across all sites sampled, across all sites in the three LEB rivers in which all taxa were sampled, and across all sites in the two currently hydrologically-connected rivers in which all taxa were sampled.

	Among Rivers			**Within Rivers**			**Within Waterholes**		
Taxa	**% Var**	F_{CT}	***P***	**% Var**	F_{SC}	***P***	**% Var**	F_{ST}	***P***
All LEB Rivers									
Goby	74.82	0.748	<0.001	1.69	0.067	<0.001	23.49	0.765	<0.001
Hardyhead	45.56	0.456	<0.001	2.43	0.045	<0.001	52.01	0.480	<0.001
Grunter	42.74	0.427	<0.001	0.75	0.013	0.804	56.51	0.435	<0.001
Perch	31.17	0.312	<0.001	2.57	0.037	<0.001	66.26	0.337	<0.001
Herring	17.96	0.180	<0.001	4.17	0.051	<0.001	77.87	0.221	<0.001
Rivers with all taxa sampled (Finke, Neales and Georgina-Diamantina rivers)									
Goby	79.39	0.794	<0.001	1.44	0.070	<0.001	19.18	0.808	<0.001
Hardyhead	46.04	0.460	<0.001	1.06	0.020	<0.001	52.90	0.471	<0.001
Grunter	42.74	0.427	<0.001	0.75	0.013	0.804	56.51	0.435	<0.001
Perch	28.69	0.287	<0.001	1.75	0.025	<0.001	69.56	0.304	<0.001
Herring	14.30	0.143	<0.001	3.30	0.038	<0.001	82.40	0.176	<0.001
Hydrologically-connected rivers with all taxa sampled (Neales and Georgina-Diamantina rivers)									
Goby	18.17	0.182	<0.001	4.57	0.056	0.012	77.25	0.227	<0.001
Hardyhead	2.04	0.020	0.094	2.93	0.030	0.005	95.03	0.050	<0.001
Grunter	5.69	0.057	0.007	0.54	0.006	0.731	93.77	0.062	<0.001
Perch	5.51	0.055	0.033	4.61	0.049	<0.001	89.88	0.101	<0.001
Herring	0.02	0.000	<0.001	4.24	0.042	<0.001	95.75	0.043	0.459

Global F_{ST} and standardised G''_{ST} values among-rivers indicate strong structure across the LEB, with the latter ranging from 0.239 to 0.795 (Table 6). The greatest differentiation was observed in the resistant goby and the least in the resilient herring; however, values were similar among the three remaining taxa, which include resistant, resilient and intermediate strategists. Pairwise F_{ST} values indicated significant differences among populations of goby in all rivers, but generally only between those in the Finke and other rivers for the other taxa. For all taxa, differentiation among the Neales, Frome and Georgina-Diamantina rivers was substantially lower than among other river systems (Appendix 2.4 Tables 1–5).

Table 6. Global unbiased F_{ST} and G''_{ST} values across the Lake Eyre Basin, and mean within-river pairwise G''_{ST} values for each sampled river system in the Basin, calculated for five fish taxa. Only river systems with five or more individuals sampled were included.

	Global Values (Among-Rivers)		**Mean Within-River Pairwise G''_{ST}**				
Taxon	**F_{ST}**	**G''_{ST}**	**Finke River**	**Neales River**	**South-West**	**Cooper Creek**	**Georgina-Diamantina**
Goby	0.718	0.795	0.149	0.075	0.091	-	0.130
Hardyhead	0.397	0.466	0.037	0.015	0.080	-	0.220
Grunter	0.374	0.437	0.018	0.085	-	-	0.135
Perch	0.283	0.448	0.067	0.060	-	0.042	0.206
Herring	0.154	0.239	0.047	0.018	-	0.047	0.072

Population differentiation within-rivers was substantially lower than among rivers for all taxa, with mean pairwise G''_{ST} ranging from 0.018 to 0.220 (Table 6). There were no few consistent differences in within-river differentiation among river systems, although mean values were greater in rivers that were sampled across a greater spatial scale (e.g. Georgina-Diamantina for most taxa). Pairwise F_{ST} and G''_{ST} values showed similar patterns, with strong differentiation among waterhole-scale populations of goby, but not the other taxa, except among the most distant sites (Appendix 2.4 Tables 6–15).

Significant Isolation By Distance (IBD) was found for all taxa within the Finke River, and within and among the hydrologically-connected Neales and Georgina-Diamantina rivers (Figs. 8–9, Appendix 2.4 Table 16). In both analyses, the resistant goby showed a very strong pattern of IBD, with a slope approximately one to two orders of magnitude steeper than those of other taxa (Fig. 8). The four other taxa showed very weak IBD, over hundreds (and even thousands) of kilometres, and exhibited very similar slopes, despite the analyses having been conducted over different spatial extents.

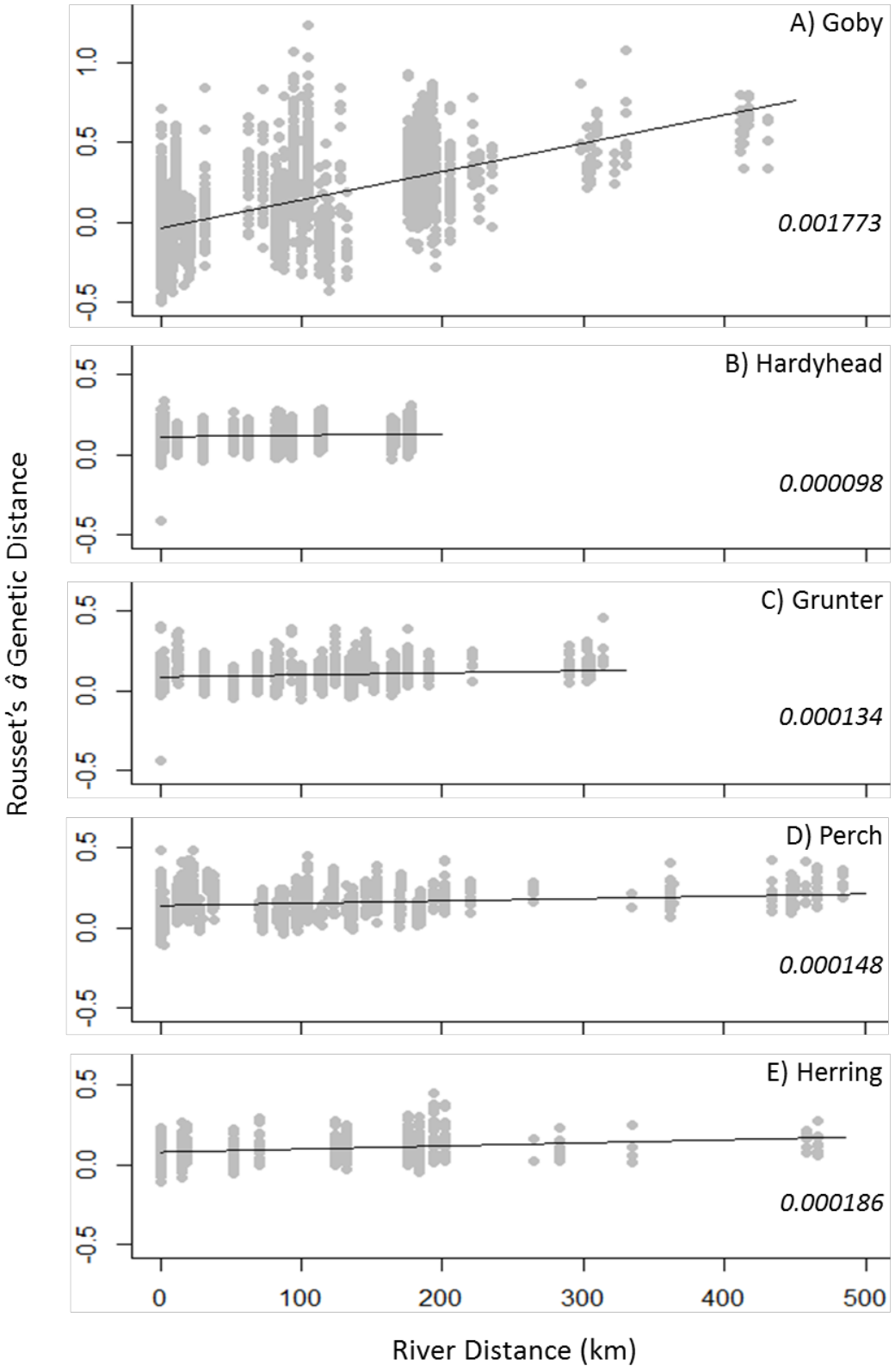


Figure 8. Scatterplots of individual pairwise genetic distances (Rousset's *â*) and river distances (km) within the Finke River for five fish taxa: A) desert goby; B) Lake Eyre hardyhead; C) barred grunter; D) spangled perch; and E) bony herring. The slope of the regression is displayed in italics.

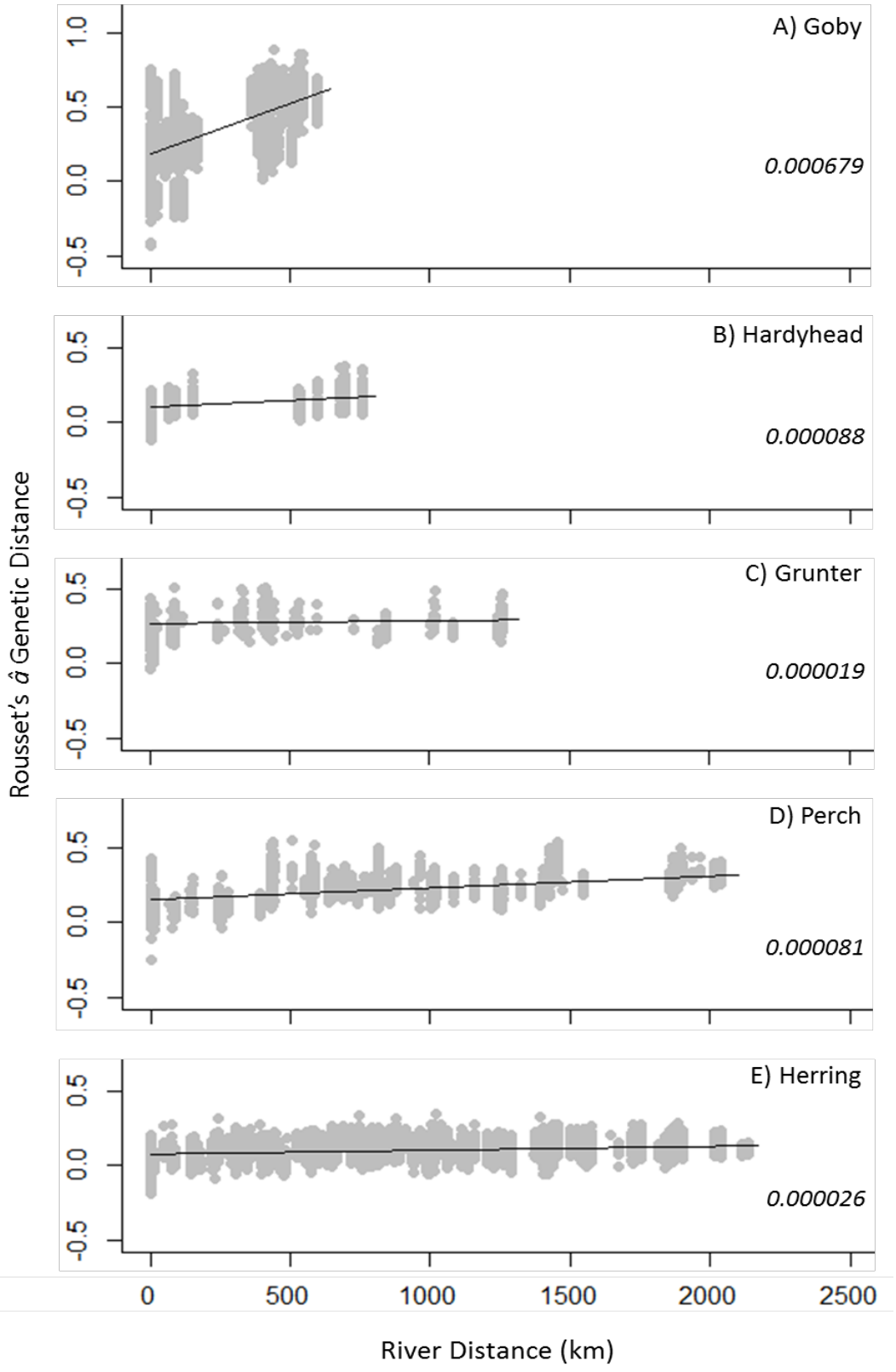


Figure 9. Scatterplots of individual pairwise genetic distances (Rousset's *â*) and river distances (km) across the hydrologically-connected Neales and Georgina-Diamantina rivers for five fish taxa: A) desert goby; B) Lake Eyre hardyhead; C) barred grunter; D) spangled perch; and E) bony herring. The slope of the regression is displayed in italics.

Contemporary Connectivity of Fish Populations of the Lake Eyre Basin

Potential first-generation, among-river migrants were detected for all five taxa with GENECLASS2 (Table 7). The number of potential migrants was low, with 0.6–4.4% of individuals more likely to have originated in a different river to that they were sampled in. No migrants of any taxon were detected in the Finke River, Cooper Creek or South-West Creeks, with most migrants of all five taxa originating in the Neales River and migrating to the Georgina-Diamantina River (Table 7). While the numbers of migrants did not correlate with persistence strategy, the distance of dispersal did. All migrants of resistant taxa were found in downstream sites close to Lake Eyre, while migrants of the intermediate taxon and one resilient taxon were also found in sites far upstream (Table 7).

Table 7. Results of migrant detection analyses conducted among rivers of the Lake Eyre Basin for five fish taxa, showing individuals identified as first-generation migrants, the river and site they were sampled in, and the most likely source river system. Results of likelihood tests also shown, including LR: likelihood ratio (L_home / L_max); *P*: *p*-value from GENECLASS test for detection of first-generation migrants; and likelihood (-log(L)) of each sampled river (bold indicates most likely source).

	Sampled		Source			Likelihood of Sources (-log(L))					
Taxon	**River**	**Site**	**River**	**LR**	***P***	**Finke**	**Neales**	**S-W**	**Frome**	**Cooper**	**G-D**
Goby											
	Geo-Dia	G16a	Neales	13.741	*0.003*	232.10	**31.86**	178.76	44.13	-	45.60
	Geo-Dia	G16a	Neales	13.757	*0.003*	234.79	**32.09**	177.04	46.57	-	45.85
	Geo-Dia	G16a	Neales	16.044	*0.001*	221.89	**29.79**	168.17	47.42	-	45.83
	Geo-Dia	G16a	Neales	8.657	*0.010*	232.27	**26.43**	160.10	39.40	-	35.09
	Geo-Dia	G16a	Neales	11.066	*0.004*	239.09	**28.53**	180.69	41.75	-	39.59
Hardyhead											
	Geo-Dia	G15	Neales	9.552	*0.003*	124.00	**31.71**	47.27	-	-	41.26
Grunter											
	Neales	N3	Geo-Dia	2.916	*0.003*	137.05	69.94	-	-	-	**67.02**
	Geo-Dia	G6	Neales	3.850	*0.001*	118.76	**61.15**	-	-	-	64.99
	Geo-Dia	G16b	Neales	4.700	*0.000*	157.61	**75.27**	-	-	-	79.97
Perch											
	Geo-Dia	G16a	Neales	8.708	*0.000*	115.73	**59.98**	-	-	116.42	68.69
Herring											
	Frome	Fr3	Neales	9.339	*0.002*	100.08	**54.21**	-	63.55	67.09	57.35
	Geo-Dia	G4	Neales	1.549	*0.005*	62.08	**33.12**	-	36.70	42.69	34.67
	Geo-Dia	G11	Neales	1.093	*0.005*	103.90	**56.10**	-	60.34	67.00	57.19
	Geo-Dia	G11	Neales	4.841	*0.001*	96.81	**53.08**	-	60.73	66.41	57.92
	Geo-Dia	G11	Neales	4.612	*0.000*	93.23	**50.80**	-	59.23	63.91	55.41
	Geo-Dia	G11	Neales	1.958	*0.004*	66.57	**35.72**	-	37.89	42.18	37.68
	Geo-Dia	G14	Neales	3.550	*0.002*	88.12	**50.49**	-	56.46	64.88	54.04
	Geo-Dia	G16a	Neales	1.307	*0.004*	103.99	**53.55**	-	61.03	64.75	54.85
	Geo-Dia	G16a	Frome	1.017	*0.004*	87.16	50.89	-	**48.45**	58.23	49.47
	Geo-Dia	G12	Frome	6.988	*0.000*	79.75	52.45	-	**49.08**	58.89	56.07

The among-rivers analyses of contemporary migration using BayesAss showed consistent estimates from independent runs, and good mixing of the Markov chain. Most individuals of all taxa were non-migrants (i.e. not migrants, or descendants of migrants in the previous two generations), with a mean percentage of non-migrants of 90.8% (range = 68.2–98.8%; Fig. 10). As with the first-generation migrant detection, gene flow was only detected among river systems that flow into Lake Eyre North, with no recent gene flow to or from the Finke River, Cooper Creek or South-West Creeks (Fig. 10). In contrast with those analyses however, recent migration was detected for only some taxa, with levels of recent gene flow among rivers not significantly different to zero for either grunter or perch. Significant levels of recent gene flow were estimated for goby, hardyhead and herring. Overall, estimates of gene flow among rivers were not correlated with persistence strategy.

Within-rivers, recent gene flow analyses also showed most individuals to be non-migrants, although estimated levels of migration were generally higher than at the among-rivers scale (see Appendix 2.5). Again, levels of gene flow within rivers were not correlated with persistence strategy, with a mean proportion of non-migrants within-rivers of 72.5%, and highly similar among taxa (range = 70.5–76.8%). The large number of migrants approaches the upper limit of migrants appropriate for BayesAss analyses, suggesting that these estimates may be inaccurate, and number of migrants may be substantially higher. Across all taxa at this within-river scale, sources of significant migration were generally limited to one waterhole within each system (see Appendix 2.5). For example, the Windorah Bridge waterhole (site C6) was a significant source of migrant herring for nine of the ten Cooper Creek waterholes sampled for this taxon. This pattern of one waterhole being a predominant source was seen in almost all river systems and taxa. The source sites were not shared among taxa, and there was no apparent pattern in spatial distribution of these sites, with sources including the most upstream and downstream sites sampled, as well as those in the middle reaches. Migrants from these sources tended to be found in many sampled sites, and not just nearby waterholes.

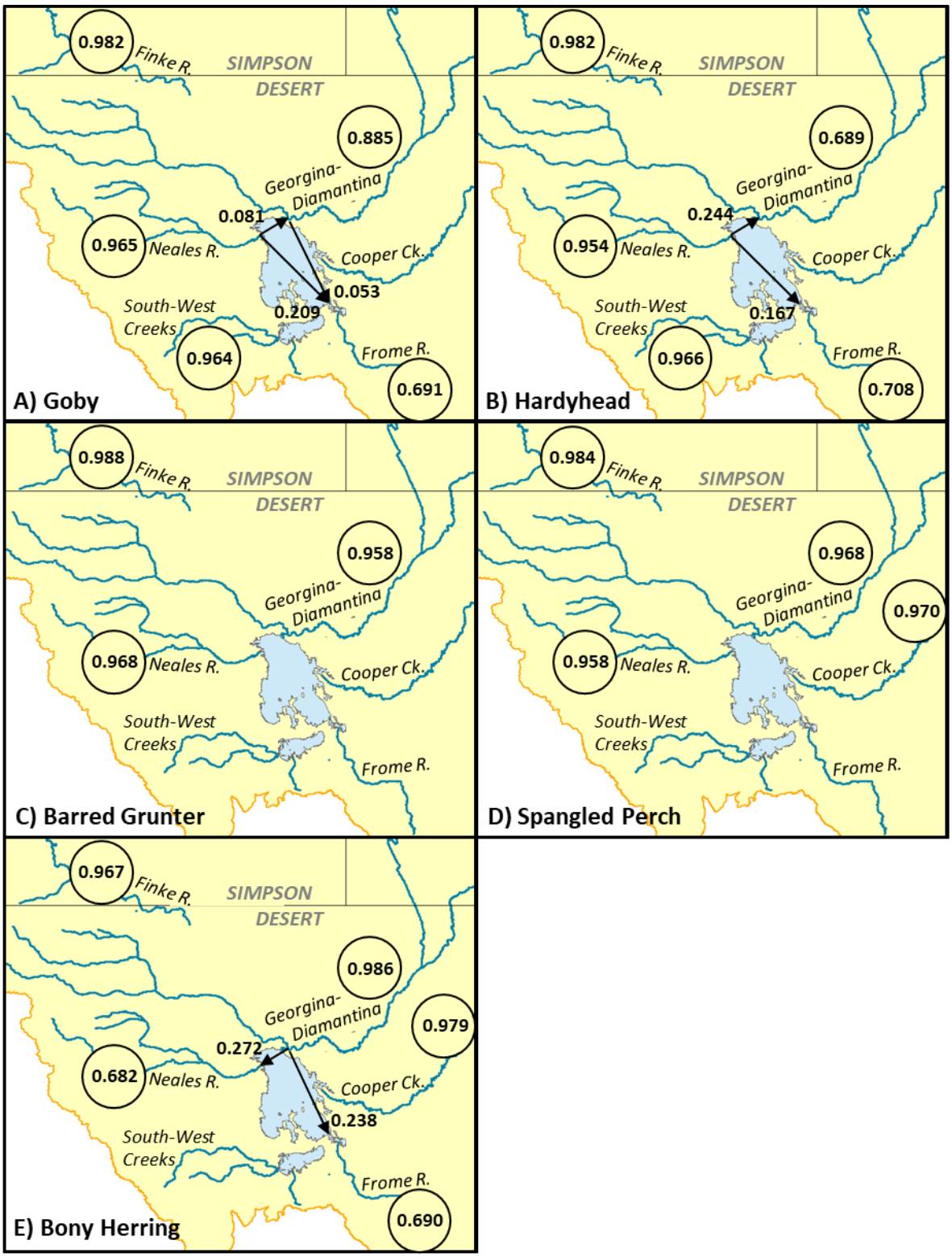


Figure 10. Recent migration rates between river systems of the Lake Eyre Basin of five fish taxa: A) Finke and desert goby; B) Finke and Lake Eyre hardyhead; C) barred grunter; D) spangled perch; and E) bony herring, as estimated by BayesAss over the last three generations. Values within circles show the percentage of non-migrants in each sampled river system. Arrows indicate the direction and magnitude of migration rates that are significantly greater than zero.

Discussion

In this study, the population genetic structure of five desert fishes in the Lake Eyre Basin (LEB) was investigated to determine the effects of alternative persistence strategies on population connectivity. Resilience-strategists, spangled perch and bony herring, exhibited relatively high population connectivity as expected, and the intermediate-strategist barred grunter showed similar patterns. The two resistance-strategists showed contrasting patterns: hardyhead had relatively high population connectivity (similar to resilient and intermediate strategists), while goby exhibited relatively low population connectivity. These patterns were consistent within and among river systems, and, as predicted, all taxa showed lower population connectivity at the greater scale. Overall, the population connectivity of desert fishes was only partially explained by persistence strategies, and other factors appear to also be important in determining population connectivity. Here, these results are discussed, with consideration of genetic patterns within and among rivers of the LEB, and of the consequences of different strategies for population connectivity and persistence of desert fishes.

Influences of Persistence Strategies on Population Connectivity within Rivers

Within river systems of the LEB, there were consistent differences in population connectivity that correlated with, and were likely driven by, persistence strategy for four of the five taxa (Table 1). The resilient taxa (perch and herring) showed very little population structure within rivers, as expected, with populations in smaller rivers panmictic. The intermediate strategist, barred grunter, showed similar structure to resilient taxa, and also exhibited panmixia. The two resilient taxa exhibited weak genetic structure that followed the Stream Hierarchy Model (SHM; Meffe & Vrijenhoek 1988) within the largest LEB river systems (the Georgina-Diamantina River and Cooper Creek, in which sample sites (for these two taxa only) were separated by up to 2,000 km; Fig. 1). This model predicts partitioning of genetic diversity according to the dendritic structure of the river network (Hughes *et al.* 2009). The resistant goby showed strong population structure within all rivers, and exhibited patterns that followed the SHM, even across relatively small spatial scales (few hundreds of kilometres) at which all other taxa were panmictic. Unexpectedly, the final resistant taxon, hardyhead, showed low structure, and exhibited panmixia.

These differences in genetic structure largely reflect the different strategies these fishes utilise for contemporary persistence. Within-rivers, persistence of fishes relies on a balance between extinction and recolonisation of subpopulations in spatially and temporally variable waterholes (Fagan *et al.* 2002; Datry *et al.* 2017). The low structure observed in resilience-strategists is expected for taxa that rely heavily on dispersal to maintain many subpopulations to ensure persistence, and thus have high population connectivity (Murphy *et al.* 2015). During floods, resilience-strategists disperse widely, potentially over hundreds of kilometres, to recolonise subpopulations, many of which will be lost during droughts (Baguette *et al.* 2013). This dynamic extinction-recolonisation process may erase much of the genetic structure among subpopulations (Woods *et al.* 2010). In contrast, resistant taxa experience lower rates of subpopulation extinction and recolonisation, and this lower population connectivity results in the accumulation of genetic structure (Frankham *et al.* 2017). While the resistant goby followed predictions, the hardyhead exhibited higher population connectivity than expected across most measures and contexts, suggesting that this taxon is more mobile than previously thought. Hardyhead have been identified as conservative dispersers in the LEB, the same category as barred grunter (Kerezsy *et al.* 2013). While dispersal ability is not the only trait included within a species' persistence strategy, it may be relatively more important for hardyhead population connectivity than other traits (e.g. environmental tolerance). Given that hardyhead have population connectivity similar to grunter and the resilient strategists, it is possible that their persistence strategy is closer to resilience than resistance (cf. McNeil *et al.* 2011b).

Levels of contemporary gene flow within-rivers were high within all LEB rivers, as expected in these dynamic river systems in which recolonisation is key for persistence. The high estimates of gene flow levels were not differentiated among taxa, likely due to limitations of BayesAss in high gene flow and/or low structure populations (Meirmans 2014). While differences in gene flow among species are anticipated, given the differences in persistence strategies, dispersal ability (Table 2; for further details see Chapter Four) and genetic structure, all taxa show evidence of dispersal over large distances within rivers. This is likely to be facilitated by large rainfall events and the low gradients of the region's rivers, which cause large, yet slow-moving, floods through which fish can disperse (McMahon *et al.* 2008b). Previous observational studies have shown that LEB fishes can undertake movements of significant distances (Marshall *et al.* 2016). For example, waterholes in a

previously dry LEB tributary were recolonised by at least twelve fish species following flow events, with some species dispersing up to 300 km upstream, including the resistant hardyhead (Kerezsy *et al.* 2013). Such long-distance dispersal is less common among many resistant fishes in deserts globally (e.g. Tibbets & Dowling 1996; Hughes *et al.* 2012; Murphy *et al.* 2015). This difference may be due to the greater temporal and spatial variability in flows in the LEB than elsewhere (Van Etten 2009; see Chapter Three for further details), which requires fishes to recolonise more often to persist in the face of higher subpopulation extinction rates.

Longitudinal patterns in levels of genetic diversity were not detected within any LEB rivers. This contrasts with the observation of greater genetic diversity in downstream fish populations than in upstream ones in many other rivers (e.g. Hänfling & Weetman 2006; Deiner *et al.* 2007; Barson *et al.* 2009). Such a pattern is typically driven by asymmetrical dispersal, with high levels of downstream drift of larval and juvenile fishes, and relatively less upstream dispersal by adults (Morrissey & de Kerckhove 2009). This suggests that other processes are dominant in the LEB, potentially driven by greater dispersal of adults in this dynamic system than are seen in more stable rivers in mesic regions or indeed other desert rivers. It is possible that 'empty' habitats, uninhabited by other fish, facilitate establishment of subpopulations, and the likely greater proportion of empty habitats within the dynamic LEB than in other basins therefore enables higher levels of successful dispersal here. Other potential drivers of differences in diversity levels are site-specific, with greater diversity expected in larger permanent refugial waterholes than in smaller ones that experience more frequent drying events (Davis *et al.* 2013). Permanent waterholes tend to be more commonly located in upstream reaches of LEB rivers, where greater topography provides some protection (via shading) against evaporation (Fensham *et al.* 2011). However, as differences among waterholes were not detected here, it is possible that such effects are masked by the dynamic extinction and recolonisation processes of LEB fishes.

Influences of Persistence Strategies on Population Connectivity among Rivers

All taxa showed greater population genetic structure among rivers than within rivers, following the expectations of lower population connectivity at larger scales validated empirically in a meta-analysis of desert aquatic systems (Murphy *et al.* 2015). The resistant goby showed much stronger genetic structure among rivers than did resilient and

intermediate taxa, although again the hardyhead showed patterns most similar to the intermediate strategist. Overall, however, these patterns were weaker than predicted for all taxa (Table 1). Resistant goby showed isolation by distance among some rivers, rather than the expected genetic isolation among all rivers, while other taxa showed weak isolation by distance and lower differentiation among rivers. While there were some river systems that appear to be genetically isolated for all taxa (see following section), all taxa showed gene flow across Lake Eyre, indicating that resistance and resilience strategists undertake substantial movements between river systems in the LEB.

Among-rivers, resistant taxa were expected to follow the Death Valley Model (DVM) of connectivity, where populations are genetically isolated from one another (Meffe & Vrijenhoek 1988). However, resistant strategists showed evidence of incomplete isolation among rivers, with patterns consistent with Isolation by Distance (IBD), at least among rivers that are hydrologically-connected (i.e. excluding the Finke River). This suggests that resistant taxa experience higher gene flow than previously thought, and may be somewhat more 'resilient' than resistant taxa elsewhere. While the DVM has been found to apply to aquatic invertebrates in springs of the LEB (Murphy *et al.* 2013), it has never been identified for LEB fishes (Hughes *et al.* 2013). The DVM was developed to explain the structure of desert pupfishes in North America (Meffe & Vrijenhoek 1988), which may represent a relatively rare case. Previous work indicates that strong genetic structure resulting from restricted gene flow is likely to be a more common pattern in resistant desert fish than is complete isolation (Murphy *et al.* 2015). This low population connectivity would allow fishes to recolonise subpopulations, negate some adverse genetic impacts of isolation, and allow beneficial alleles to spread throughout the population (Lowe & Allendorf 2010).

Recent migration of all taxa was identified among several river systems that are hydrologically connected via Lake Eyre North (Fig. 1; Kotwicki 1986). Lake Eyre has been considered a barrier to fish dispersal (Unmack 2001a). For the lake to fill, an event that happens on an approximately decadal timescale, multiple river systems must flood simultaneously (Leon & Cohen 2012). However, as it fills, a thick salt crust dissolves, and salinity increases to levels greater than seawater (Habeck-Fardy & Nanson 2014). Accordingly, dispersal via Lake Eyre is only possible when at least two rivers flow into it for long enough that fish may migrate upstream to suitable habitat, but before the waters of

the Lake become too saline for fishes to tolerate (Unmack 2013). Previous studies using traditional genetic markers have not detected migration across Lake Eyre (e.g. Huey *et al.* 2008; Masci *et al.* 2008; Huey *et al.* 2011a). However, population genomic approaches offer greater power to detect contemporary movements (Allendorf *et al.* 2010). Here, they demonstrate that all fishes dispersed during the most recent fill event prior to sampling (in 2009–2011, Backway 2014), enabling population connectivity among rivers.

While persistence strategy does not explain the differences in recent migration rates observed among fish taxa, other aspects of species' ecology do. The three taxa that exhibit higher recent gene flow (goby, hardyhead and herring) can tolerate greater levels of salinity (>50 ‰) than the grunter and perch (<50 ‰; McNeil *et al.* 2011b; for more details on salinity tolerances see Chapter Four). This greater tolerance may have enabled the successful dispersal of goby, hardyhead and herring across the Lake during the most recent opportunity. Salinity is known to structure desert fish communities (Higgins & Wilde 2005); however, within the LEB, it does not appear to be a major determinant of population connectivity. Despite the low recent gene flow observed for grunter and perch among the Neales, Frome and Georgina-Diamantina, all five taxa exhibited relatively low population differentiation among these rivers, suggesting this contemporary result may not be representative of long-term levels of gene flow. It is clear that Lake Eyre is a less significant barrier than previously thought, with other recent studies indicating connectivity across the lake for other fishes (e.g. Faulks *et al.* 2010b; Beheregaray & Attard 2015). Overall, recolonisation of river systems following a hypothetical river-wide extirpation event appears to be possible for resistant and resilient taxa in the LEB.

Influences of Structural Connectivity on Population Connectivity among Rivers

Population connectivity is strongly influenced by structural connectivity, i.e. environmental variables, which for desert fishes include river geomorphology and hydrology (Hughes *et al.* 2013). This study indicates that diverse species, with varying persistence strategies, are affected in similar ways by the LEB's structural connectivity. For all five taxa, there was strongest differentiation between populations in the hydrologically-disconnected Finke River and the rest of the LEB rivers, with zero gene flow detected. This suggests that Finke fish populations are isolated, a pattern noted in several previous studies (Unmack & Dowling 2010; Adams *et al.* 2013; Mossop *et al.* 2015). Isolation is expected to impact persistence of

desert fish populations, with taxa that rely more heavily on structural connectivity (i.e. resilience-strategists) likely to be more strongly affected. While previous work has identified some ecological traits associated with desert fish vulnerability to reduced connectivity (Fagan *et al.* 2002; Olden *et al.* 2008), the impacts of isolation on fishes with different persistence strategies has not been assessed.

Among the rivers of the LEB that are not hydrologically isolated (i.e. those that do connect to Lake Eyre), differences in structural connectivity related to frequency of hydrological connection also appear to impact fish populations. Populations in the South-West Creeks, which drain into Lake Eyre South, were differentiated from populations in river systems that drain into Lake Eyre North. The two lakes connect via the Goyder Channel, but this is not known to have filled since 1989 (Kotwicki 1986; Habeck-Fardy & Nanson 2014). Accordingly, fish populations in the river systems of Lake Eyre South may be largely isolated from those in the river systems of Lake Eyre North. Given the substantial unique diversity observed in the South-West Creeks populations of desert goby and Lake Eyre hardyhead, these populations may have been divergent for a significant period of time and be locally-adapted, as suggested in previous studies (Unmack & Dowling 2010; Mossop *et al.* 2015).

The fish populations of Cooper Creek also appear to experience restricted population connectivity due to lower structural connectivity compared to the other rivers of Lake Eyre North, a pattern previously detected for several species, including resistant and resilient taxa (e.g. Huey *et al.* 2008; Masci *et al.* 2008; Faulks *et al.* 2010b; Huey *et al.* 2011a; Beheregaray & Attard 2015). Restricted connectivity is also suggested by the Creek's unique fish assemblage, which includes an endemic species and the absence of several otherwise-widespread species, such as barred grunter (Wager & Unmack 2000). Cooper Creek appears to experience hydrological connections to Lake Eyre North on similar timescales to other rivers (Kotwicki 1986), indicating that hydrology is unlikely to reduce population connectivity. It is possible that environmental conditions in Cooper Creek are different to those elsewhere, resulting in different selective pressures that may prevent successful gene flow into and out of the Cooper (Beheregaray & Attard 2015). Further investigation of the structural connectivity and environment of Cooper Creek would be useful for understanding its distinctive fish fauna.

Implications of Persistence Strategies for the Future Persistence of Desert Fishes

The future persistence of species in changing environments relies on their ability to either move to more suitable conditions, or to adapt to new conditions *in situ* (Sgro *et al.* 2011). For LEB fishes, and many desert fishes globally, moving to new areas will not be possible given the lack of hydrological connections between basins (see Chapter Three for further details). Therefore, their future persistence depends on their ability to adapt, i.e. their evolutionary potential (Frankham 2012). As evolutionary potential is partially determined by a population's genomic diversity, those with greater diversity should have greater potential for future persistence (Harrisson *et al.* 2014). Fragmented populations contain lower levels of diversity than those in continuous habitats (Reid *et al.* 2008), and it is therefore expected that desert fishes will have lower evolutionary potential than do fishes in more mesic regions. Given that greater population connectivity is expected to maintain genetic diversity more effectively, resilient taxa should be more able to persist into the future. However, this requires the maintenance of population connectivity, which may not occur if future environmental changes increase fragmentation. In such cases, resistant taxa, which are expected to harbour greater diversity within populations and subpopulations, will have greater evolutionary potential and therefore prospects of future persistence.

For resistant and resilient fishes, maintenance of refugial habitats (permanent waterholes) and structural connectivity (flow events) is vital for population connectivity and therefore contemporary and future persistence. However, while both strategies require population connectivity, resistant taxa are likely to be less vulnerable to reduction or loss of connectivity, especially among rivers, given they already experience restricted population connectivity (Phillipsen *et al.* 2015). However, resistance-strategists are also expected to harbour a greater level of unique diversity in a given river than resilience-strategists, and so in a river-wide extinction event are likely to lose unique variation. Because of this, the appropriate scale of management differs depending on strategy (Toro & Caballero 2005); resistant taxa should be managed at the river scale, and resilient taxa at the basin scale. This would allow preservation of unique diversity within rivers (such as that observed in the resistant goby populations in the South-West Creeks of the LEB) and natural population processes (such as widespread gene flow among rivers for resilient taxa). This study indicates that determination of a species' persistence strategy may be complex, and that observations of responses to single events may not provide sufficient information on which to base

conclusions. However, given these strategies are likely to be widely utilised by desert fishes, if strategies can be reliably identified (and confirmed via genetic analyses), management approaches based on strategy are likely to be useful in many desert freshwaters globally.

Conclusions

The study of suites of diverse species inhabiting a single region allows the exploration of the effects of different ecological traits on species' evolutionary processes. Here, the consequences of persistence strategies for the population connectivity of desert fishes were established for the first time. Strategy was a strong predictor of population connectivity within rivers for most taxa, but other factors (including specific aspects of species' ecology and structural connectivity) were also important when considering the among-rivers scale. Resilient and resistant strategists were both effective at maintaining population connectivity, and contemporary and future persistence, in this extreme and fragmented desert river system. Future persistence is likely to be similar among resistant and resilient taxa, although resilience strategists are likely to be less able to respond to future environmental change if structural connectivity is lost. These findings are likely applicable to desert fishes in arid regions globally. Overall, persistence strategies provide new insights into our understanding of how desert fishes persist and offer a new way to expand our knowledge of persistence in fragmented environments.

Chapter Six

Divergence in the Desert: The Impact of Isolation and Ecology on Genetic Diversity and Divergence of Fishes in an Ancient River

Abstract

Anthropogenic environmental change is expected to isolate many populations through expansion of unfavourable habitat. To understand the evolutionary genetic consequences of population isoaltion, natural experiments where populations of diverse, co-distributed species have been disconnected by the same process can be studied. In central Australia, late Quaternary aridification led to hydrological disconnection of the Finke River from other rivers of the Lake Eyre Basin. This resulted in population isolation of fish species possessing different suites of traits defining persistence strategies: resistance (high tolerance of environmental extremes, but low mobility) and resilience (the converse). We tested whether complete isolation of the Finke led resistant species to experience smaller reductions in effective population sizes and loss of genetic variation than resilient species, which have higher reliance on mobility and less on hardiness. We investigated isolation-with-migration coalescent histories of five species using >700 nuclear loci sequenced using MetaPrep, a novel sequencing approach that allows samples from different species to be pooled before sequencing. A wide range of estimated onsets of isolation of Finke populations (~50,000 to ~3,000 years ago) did not contradict the estimated hydrological disconnection of the Finke, but were unrelated to persistence strategy. Emigration and immigration rates for the Finke since onset of divergence were positively associated with measures of genetic variation and persistence strategy. Finke coalescent effective population sizes were unrelated to persistence strategy. We conclude that both persistence strategies should be able to retain genetic diversity, when some gene flow is present. This work illustrates the value of genomic coalescent estimates of population parameters to understanding the causes and consequences of population isolation.

Introduction

Climatic changes during the Pleistocene caused population fragmentation and isolation across a wide range of species globally. In the Northern Hemisphere, these isolations were largely caused by glaciation restricting populations to refugia. Many of these populations have since experienced reconnection (Hewitt 1996; Knowles 2001; Gante *et al.* 2009; April *et al.* 2013). In contrast, Australia remained almost completely unglaciated; instead, population isolation in many species across the continent was driven by aridification, which was most extreme during the Last Glacial Maximum (LGM), ~21 kya (thousand years ago; Hesse & Simpson 2006; Byrne *et al.* 2008). Despite milder interglacial periods, including a temporary return in some areas to wetter climate in the interglacial after the LGM (Luly 2001), aridity subsequently returned and has persisted to the present, with each interglacial being more arid than the one before (Martin 2006; Byrne *et al.* 2008; Fitzsimmons *et al.* 2013). Central Australia provides an opportunity to investigate the genetic and evolutionary consequences of population isolation caused by aridification.

Aridification has an exceptionally strong impact on freshwater ecosystems. As well as reducing hydrological connectivity, it poses challenges related to the intermittent or ephemeral nature of freshwater habitats, with long periods of high salinity, high temperature, and other extreme conditions affecting aquatic biota (Stanley *et al.* 1994; Kingsford 2006). During droughts in arid regions, obligate aquatic taxa, including fish, are often restricted to a small number of remnant waterholes (Arthington *et al.* 2005; Robson *et al.* 2013). These waterholes rarely provide suitable environments for all species; as water levels recede, habitat and food become scarce, competition and predation increase, and water quality declines (Sheldon *et al.* 2010; McNeil *et al.* 2015). Consequently, some species may decline in numbers at multiple spatial scales, become restricted to fewer waterholes or even go extinct within a river system (McNeil *et al.* 2011b; Davis *et al.* 2013; Murphy *et al.* 2015).

Species that persist in intermittent arid river systems exhibit different suites of life history traits comprising a range of persistence strategies (Stanley *et al.* 1994; McNeil *et al.* 2011a; McNeil *et al.* 2015). A resistance strategy characterises species that survive in small pools during dry periods, even as environmental conditions deteriorate, whereas a resilience strategy characterises species that may become locally extinct under harsher environments, but maintain populations in larger pools with more stable conditions, from which individuals

recolonise more widely after flows resume (Lake 2000; Nimmo *et al.* 2015). Persistence strategies have been attributed to fish species based on observational studies of short-term responses to flow events (e.g. McNeil *et al.* 2011b; Marshall *et al.* 2016). Aridification is expected to have different effects on fishes with different strategies. Compared to resistance-strategists that can survive environmental extremes, resilience-strategists, which rely on hydrological connectivity, might experience greater loss of genetic connectivity and be at greater risk of loss of genetic diversity and local extinction (Robson *et al.* 2011; Phillipsen *et al.* 2015).

Central Australia's Lake Eyre Basin (LEB) is the world's largest internally-draining basin (Fig. 1). Within the LEB, the main aquatic habitats are perennial groundwater-fed springs, and a network of ephemeral rivers that usually exist as series of isolated waterholes (McMahon *et al.* 2008b). One of the greatest impacts of aridification on the LEB was the formation and expansion of the Simpson Desert, which disconnected several rivers from Lake Eyre, the last being the Finke River, one of the world's oldest rivers (Wasson 1984; Craddock *et al.* 2010). The Finke's disconnection from the Macumba River occurred approximately 10–20 kya (Kotwicki 1989; Unmack 2001b). Finke populations of aquatic taxa are now separated from other populations by several hundred kilometres of desert, into which the river dissipates via 'floodout' areas (Duguid 2005). Flows have never been recorded beyond the last floodout, which is more than 80 km from the nearest part of the Macumba catchment, even in the largest flood for 850 years, in 1974 (Williams 1970; Pickup 1991; Duguid *et al.* 2005). The Finke has nine species of native fish, with a range of persistence strategies, although none has any desiccation-resistant life-stages or the ability to aestivate, despite anecdotal suggestions otherwise (Wager & Unmack 2000; McNeil *et al.* 2011b). As such, the fish are restricted to refuge waterholes separated by up to many tens of kilometres of dry riverbed, reconnected by sporadic flows that do not occur every year.

The disconnection of the Finke River from the wider LEB provides an opportunity to investigate whether the long-term genetic consequences of isolation on fish species are influenced by their persistence strategy, as would be expected from different reliance on gene flow and resistance to harsh conditions. Resilience-strategists rely on gene flow for persistence and are not strongly resistant to the harsh environmental conditions imposed by extremely dry periods. We hypothesised that following cessation of gene flow due to

aridification, resilience-strategists in the Finke River would be subject to stronger population size decline and loss of genetic variation through drift than would be experienced by resistance-strategists. We test this hypothesis using two complementary approaches.

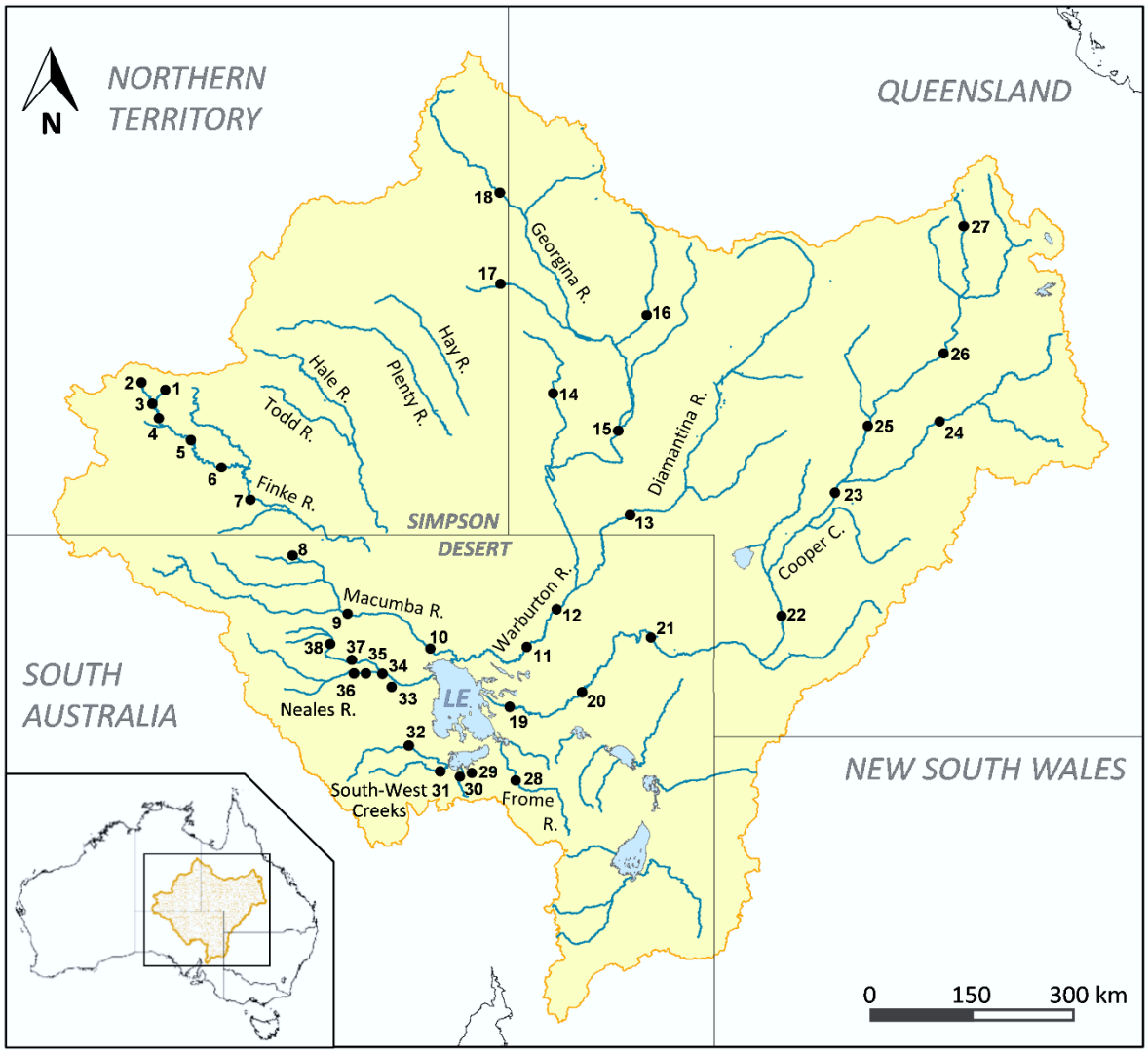


Figure 1. Sampling locations (dots) on the Finke River (sampling locations 1–7) and other rivers of the Lake Eyre Basin. The waterbody labelled LE is Lake Eyre. For a detailed list of sites and samples, see Appendix 3.1.

First, we establish the demographic history of divergence of fish populations between the Finke and wider LEB, using the isolation-with-migration model to estimate time of population divergence, gene flow since divergence, and effective population sizes of ancestral and descendent populations (Hey 2010). Assuming complete disconnection of the Finke River occurred rapidly and resulted in zero gene flow, and that Simpson Desert expansion was the major cause of population divergence, we predict that populations of the five species diverged approximately simultaneously. Gene flow-dependent resilience-

strategists would be expected to experience greater relative declines of effective population size in the Finke than the environmentally-tolerant resistance-strategists, because resistant taxa are able to persist in more sites during drought periods (as shown by McNeil *et al.* 2011b). However, if the expansion of the Simpson Desert did not rapidly and completely disconnect the Finke from the wider LEB, populations in each may have still been connected by gene flow during divergence. In this case gene flow would be expected to be higher for resilience-strategists. The effects on effective population sizes would differ according to levels and timing of fish movements and gene flow, and subsequent demographic processes.

Second, we compare the levels of loss of genetic diversity in the Finke population relative to wider LEB populations, across species of different persistence strategies. We assume that the LEB populations represent the levels of genetic diversity that would be seen in the Finke had it not been disconnected, as both have experienced similar environmental pressures. Under a scenario of complete and rapid disconnection of the Finke, there should be stronger loss of diversity for resilience-strategists, owing to their relative susceptibility to environmental extremes and fluctuating population sizes, leading to stronger genetic drift. We are interested in genetic diversity as a separate measure from coalescent effective population size, because the correlation between the two can be reduced by many factors of evolutionary history, and genetic diversity is an important predictor of adaptive potential (Frankham 2012; Harrisson *et al.* 2014). These predictions regarding genetic diversity may not hold if divergence times are not simultaneous or in the presence of gene flow, which can affect levels of variation.

To test these predictions for five fishes with contrasting persistence strategies, we used a novel sequencing approach. It samples anonymous nuclear loci across the genomes of multiple species simultaneously, yielding a dataset of >700 nuclear DNA sequences for each taxon. Data for each species were used to estimate parameters of the isolation-with-migration model and to estimate genome-wide diversity. An improved understanding of the evolution and genetic diversity of ecologically-diverse taxa affected by aridification will inform the development of predictions about species persistence under ongoing environmental change.

Methods

Study Taxa, Sampling and Molecular Data Collection

Seven endemic Australian fish species were included in this study (Table 1). Three are common and widespread across much of central and northern Australia, and occur across the LEB (bony herring, *Nematalosa erebi*; spangled perch, *Leiopotherapon unicolor*; and barred grunter, *Amniataba percoides*). Two species are of more restricted distribution, endemic to the LEB: desert goby *Chlamydogobius eremius,* and Lake Eyre hardyhead, *Craterocephalus eyresii* (Wager & Unmack 2000). These latter species have recognised close relatives endemic to the Finke River: Finke goby, *Chlamydogobius japalpa* and Finke hardyhead, *Craterocephalus centralis*, both listed as species of conservation concern (Northern Territory Government 2016). For the purposes of the analyses in this paper, the pair of goby species were analysed together, as were the two hardyheads.

Table 1. Study taxa and their *a priori* persistence strategies (following McNeil *et al.* 2011b), relative dispersal ability, mean adult standard length (mm, excluding tail) in the Lake Eyre Basin (Crowley & Ivantsoff 1990; Larson 1995; Pusey *et al.* 2004; Wager & Unmack 2000), and sample sizes of each taxa collected in the Finke River and the other river systems of the wider Lake Eyre Basin (LEB). NA - not applicable: species does not occur in this location. For a detailed list of sample sites, see Appendix 3.1.

Family	Taxon	Strategy	Dispersal	SL	Finke	LEB
Gobiidae	Finke Goby *Chlamydogobius japalpa*	Resistance	Weak	50-60	66	NA
	Desert Goby *Chlamydogobius eremius*	Resistance	Weak	50-60	NA	108
Atherinidae	Finke Hardyhead *Craterocephalus centralis*	Resistance	Moderate	50-65	70	NA
	Lake Eyre Hardyhead *Craterocephalus eyresii*	Resistance	Moderate	50-100	NA	72
Terapontidae	Barred Grunter *Amniataba percoides*	Intermediate	Moderate	100-200	52	31
	Spangled Perch *Leiopotherapon unicolor*	Resilience	Strong	150-300	54	80
Clupeidae	Bony Herring *Nematalosa erebi*	Resilience	Strong	150-300	35	153

The five taxa include two resistance-strategists, two resilience-strategists and one intermediate-strategist (Table 1). Desert and Finke gobies are small-bodied resistance-strategists, able to persist in isolated waterholes with extreme environmental conditions, including in salinity three times that of seawater, extreme temperatures (e.g. 5–41 °C) and low dissolved oxygen (Glover 1971; Thompson & Withers 2002). The other resistance-strategist, Lake Eyre and Finke hardyhead, is also tolerant of environmental extremes (McNeil *et al*. 2015). Gobies and hardyheads rarely disperses large distances (McNeil *et al*. 2011a; but see Kerezsy *et al.* 2013), although this is based on research in the wider LEB, with no studies having been conducted on the dispersal ability of Finke fishes. Both resistant taxa were found to persist in numerous waterholes during an extended drought in the LEB and, when flows resumed, did not rapidly colonise newly available habitat (McNeil & Schmarr 2009). In contrast, other fishes were restricted to very few waterholes during the drought. Following resumption of flows, the two resilience-strategists, spangled perch and bony herring, were found to rapidly recolonise (McNeil & Schmarr 2009). Spangled perch is the most widespread freshwater fish in Australia, and one of the most dispersive, recorded swimming through water less than half their body depth during overland flow events (Wager & Unmack 2000). Bony herring is also a strong disperser, with a previous study documenting dispersal events over more than 300 km (Kerezsy *et al.* 2013), although a relatively low proportion of individuals are vagile (Marshall *et al.* 2016). Further, they are an environmentally-sensitive species (McNeil *et al.* 2011b), and may require high water quality for dispersal. The intermediate-strategist, barred grunter, also has a broad geographic distribution, but may not have strong long-distance dispersal ability (Kerezsy *et al.* 2013). As with the resilience-strategists, this species was restricted to few waterholes during drought, however it did not rapidly recolonise with flows, instead doing so over a longer time period (McNeil & Schmarr 2009).

The two Finke resistance-strategists, hardyhead and goby, have been distinguished from their LEB counterparts on the basis of morphological features, including scalation patterns and numbers of fin rays, considered sufficient to warrant their description as distinct species (Crowley & Ivantsoff 1990; Larson 1995). However, phylogeographic and phylogenetic studies have found genetic divergences between Finke and wider LEB forms of gobies and hardyheads to be of similar magnitude to divergence among some populations in different river systems in the LEB (Crowley & Ivantsoff 1990; Mossop *et al.* 2015; Unmack &

Dowling 2010). No morphological distinctiveness of Finke populations of the two resilience-strategists, nor the intermediate-strategist, has been documented, although none has been subject to recent morphological study. Finke populations of bony herring show some genetic differentiation from LEB populations (Bostock 2014; Beheregaray & Attard 2015), whereas spangled perch shows very limited genetic divergence (Bostock *et al.* 2006; Bostock 2014). No phylogeographic studies of barred grunter are available.

These fishes were sampled in the Finke and other river systems (including the Neales, Frome and Georgina-Diamantina rivers, Cooper Creek, and four smaller watercourses that drain into Lake Eyre South, termed 'South-West Creeks') of the LEB, central Australia (Fig. 1, Table 1, for full details see Appendix 3.1). Additional samples were received from the collections of the South Australian Museum, Museum and Art Gallery of the Northern Territory, and Queensland Department of Natural Resources and Mines. The total number of samples obtained ranged from 83 to 188 individuals for each taxon.

Molecular data were collected and processed in the same way as in Chapter Five, generating sequence and genotype data for up to 1,000 anonymous nuclear loci per taxon. For the genotype dataset, a putatively neutral dataset was constructed by removing loci identified as putatively under selection. For complete details on the data collection, bioinformatics and selection analysis methods see Chapter Five.

Population Structure Characterisation

To examine the population subdivision across the LEB, we analysed the genotype dataset using STRUCTURE 2.3, which implements a Bayesian, individual-based non-spatial clustering algorithm (Pritchard *et al.* 2000; Falush *et al.* 2003). STRUCTURE was run using the admixture model with correlated allele frequencies, assuming from one to twelve genetic clusters (K). Twenty replicates of 1,000,000 Markov Chain Monte Carlo runs following a burn-in period of 500,000 repetitions were performed for each value of K. Results of all runs were visualised using CLUMPAK (Kopelman *et al.* 2015) and summarised using STRUCTURE HARVESTER 0.6.94 (Earl & von Holdt 2011). The summary statistic ΔK (Evanno *et al.* 2005), as well as the biologically informative structure observed (Meirmans 2015), were considered when interpreting the most likely number of clusters.

To quantify population subdivision across the LEB, we estimated the allele frequency differentiation between each of the sampled river systems (Finke and those in the wider LEB) using Nei's (1973) pairwise F_{ST} estimates calculated using the R package *Hierfstat* 2.01 (Goudet 2005). To estimate if differentiation was significant, we obtained a null-distribution of F_{ST} values by computing 1000 permuted F_{ST} matrices with individuals randomly distributed among groups. To calculate *p*-values, we determined the proportion of null-distribution estimates that were higher than observed F_{ST} values. In addition, to estimate the genetic differentiation of each population relative to all other populations, we estimated population-specific F_{ST} values using the program GESTE 2.0 (Foll & Gaggiotti 2006). Default settings were used, with ten pilot runs of 5000 iterations and an additional burn-in of 50,000 iterations, followed by 200,000 iterations (sample size of 10,000 and thinning interval of 20).

Demographic History Inferences

To estimate the divergence time, coalescent effective population sizes (N_ev) and gene flow between the Finke and the wider LEB, we used the two-population model of isolation-with-migration (Hey & Nielsen 2004; Hey 2010) implemented in IMa2p (Sethuraman & Hey 2016). As IMa2p assumes selective neutrality, the neutral sequence dataset was used. For the goby, which showed evidence of more than two distinct genetic clusters (based on STRUCTURE results), samples from South-West Creeks were omitted from IMa2p analysis, because they were previously shown to comprise a sister-taxon to that comprising Finke and other northern LEB gobies (Mossop *et al.* 2015). To make running time tractable even on a large computer cluster, we constructed datasets for each taxon that contained 25 randomly selected individuals from each of the two populations and 200 neutral loci selected randomly (i.e. not biased towards polymorphic ones). To meet the assumption of no recombination within loci since divergence, recombination points were detected for each locus using the four-gamete test implemented in IMgc (Hudson & Kaplan 1985; Woerner *et al.* 2007); the largest non-recombining fragment of each locus was used for analyses.

To optimise prior parameter boundaries for IMa2p, preliminary runs were performed. Final analyses included three replicate runs with different starting seeds, each of 256 chains and geometric heating terms of –ha = 0.9 and –hb = 0.3. M-mode runs ranged from 1–2 million steps, following a burn-in of at least 500,000 steps that was ended after trace plots for all

parameters showed no visible trends. A summarising L-mode run was conducted that included likelihood ratio tests to assess whether estimated migration rates differed significantly from zero (Nielsen & Wakeley 2001; Hey 2010). While the IM-program family can return false-positives in tests of migration in scenarios with recent divergence, this occurs primarily when datasets are small (Cruickshank & Hahn 2014; Hey *et al.* 2015) and should not be a problem for our data.

Parameter estimates were converted to demographic units using a generation time of one year for goby and hardyhead, and two years for grunter, perch and herring. Generation time, defined as average age at reproduction, was estimated based on age at sexual maturity, expected longevity, and age-class structure of each taxon (Glover 1971; Pusey *et al.* 2004). No fossils or geographic events are available to calibrate the mutation rate, and recent mutation rates based on genome-wide data are not established for fish, except for one study of desert pupfish that showed highly elevated rates, potentially due to biased genomic sampling strategy, extreme environmental conditions, and/or a very small population size (Martin *et al.* 2016). Accordingly, we chose a wide range of priors (10^{-10}–10^{-8} substitutions per site per year for goby, hardyhead and herring), encompassing rates in other vertebrate studies using genome-wide data (e.g. lizards, Leaché *et al*. 2013; birds, Lerner *et al*. 2011; Nadachowska-Brzyska *et al*. 2013; Kozma *et al*. 2016). The highest of those rates approaches the genome-wide rate found in a cichlid pedigree study (Recknagel *et al.* 2013), which is expected to be much faster than historic rates (Ho *et al.* 2011). As perch and grunter are in the family Terapontidae, it is likely that the step of identifying loci that would not cross-capture in these species would tend to preference faster-evolving loci. The frequency distribution of nucleotide diversity vs number of loci for perch and grunter differed from the other three taxa: the terapontids had few loci with low levels of diversity and a peak diversity approximately an order of magnitude greater (Appendix 3.2). Thus, we scaled up the prior range of mutation rates in grunter and perch to 10^{-9}–10^{-7} substitutions per site per year, assuming that in these species the greater diversity was due to sequence capture bias towards faster-evolving loci. This scaling was not necessary for goby and hardyhead sister-species, as they were never in the same MetaPool. As in most studies, our mutation rates are approximations because we do not have access to reliable rate calibrations for an appropriate time span, and inferred demographic values should be interpreted with caution and validated with better calibrated data when available.

Genetic Diversity Analyses

To examine patterns of neutral genetic diversity within each taxon, summary genetic statistics were calculated for each waterhole-scale sample, with sample sizes of five or more, and averaged for the Finke and wider LEB. Nucleotide diversity (π) was calculated using the R package *PopGenome* (Pfeifer *et al.* 2014). The R package *DiveRsity* (Keenan *et al.* 2013) was used to calculate allelic richness (AR), observed and expected heterozygosity (H_O, H_E) of each population, as well as the overall proportion of alleles unique to the Finke and wider LEB. To test if the diversity measures differed statistically significantly among waterholes in the Finke and wider LEB, we computed mixed effect models with locus and waterhole as random effects, and 'river' as a fixed effect (two levels: Finke and wider LEB).

To investigate the inferred effect of isolation on genetic diversity of each taxon, and test for significant differences among taxa, a meta-analytical framework was utilised to calculate effect sizes for three genetic diversity statistics: AR, H_E, π (following Blanchet *et al.* 2010b). We calculated Cohen's *d* value and its 95% confidence interval for each taxon and diversity statistic, using locus averaged over waterholes as the replicate unit (see Appendix 3.5). The Cohen's *d* value is a standardised effect size, with a value of zero indicating no inferred effect (Rosenberg *et al.* 2000), in this case of population isolation, on the genetic diversity of the 'treatment' (i.e. the Finke) compared to the 'control' (the wider LEB). Values further from zero represent a greater effect. We also calculated the weighted cumulative effect size and its 95% confidence interval for each diversity statistic. To determine whether species had homogeneous or heterogeneous effect sizes, the total heterogeneity (Q_T) was calculated and its significance tested using chi-square statistics (Koricheva *et al.* 2013).

Results

Genetic Divergence between the Finke and Wider Lake Eyre Basin Fish Populations

The exploratory STRUCTURE analyses showed relatively similar patterns of large-scale genetic structure among fish taxa in the LEB. All taxa showed strong differentiation between the Finke and the wider LEB at K=2 (Appendix 3.3), although the resilience-strategists sampled in the wider LEB exhibited some minor representation of the Finke cluster (perch: max. Q = 0.179; herring: max. Q = 0.092). The best-supported value of K was K=3 for goby

and K=2 for the other taxa. For all taxa, increasing values of K indicated further differentiation only in the wider LEB (Appendix 3.3).

The two resilience-strategists (perch and herring) had similar levels of genome-wide differentiation between the Finke and the other sampled river systems (Nei's pairwise F_{ST} range: 0.096–0.205, $p < 0.05$), and these were very similar to those of the intermediate-strategist grunter (0.051–0.208, $p < 0.05$; Appendix 3.3, Tables 1–5). The two resistance-strategists showed contrasting levels of differentiation: the hardyhead's F_{ST} (0.121–0.239, $p < 0.05$) was similar to that of resilient species, whereas values were much higher for the goby (0.604–0.795, $p < 0.001$; Appendix 3.3). Population-specific F_{ST} values show that the Finke is the most differentiated river for all five taxa, with populations in river systems in the wider LEB less differentiated from each other, especially for resilience-strategists (Table 2).

Table 2. Population-specific F_{ST} values for each sampled river system in the Lake Eyre Basin for five fish taxa. Only river systems with five or more individuals sampled included.

Taxon	Finke River	Macumba River	Neales River	South-West	Frome River	Cooper Creek	Georgina-Diamantina
Goby	0.818	-	0.327	0.318	0.476	-	0.529
Hardyhead	0.419	-	0.121	0.297	-	-	0.264
Grunter	0.417	-	0.095	-	-	-	0.089
Perch	0.357	0.173	0.153	-	-	0.260	0.099
Herring	0.266	0.109	0.064	-	-	0.139	0.091

Demographic History of Finke River Fish Populations

All coalescent simulation runs for a given taxon converged on the same posterior probabilities (Appendix 3.4). Although the most-likely estimates of population divergences do not indicate simultaneous splitting, nor a correlation with persistence strategy (Fig. 2), the locations of the confidence intervals are compatible with divergence at the time of Finke isolation 10–20 kya. Finke populations of resistance-strategist hardyhead and resilience-strategist herring were estimated to have diverged from the wider LEB in the Late Pleistocene, ~10–50 kya. Holocene divergences ~3–19 kya were inferred for resistance-strategist goby, intermediate-strategist grunter and resilience-strategist perch.

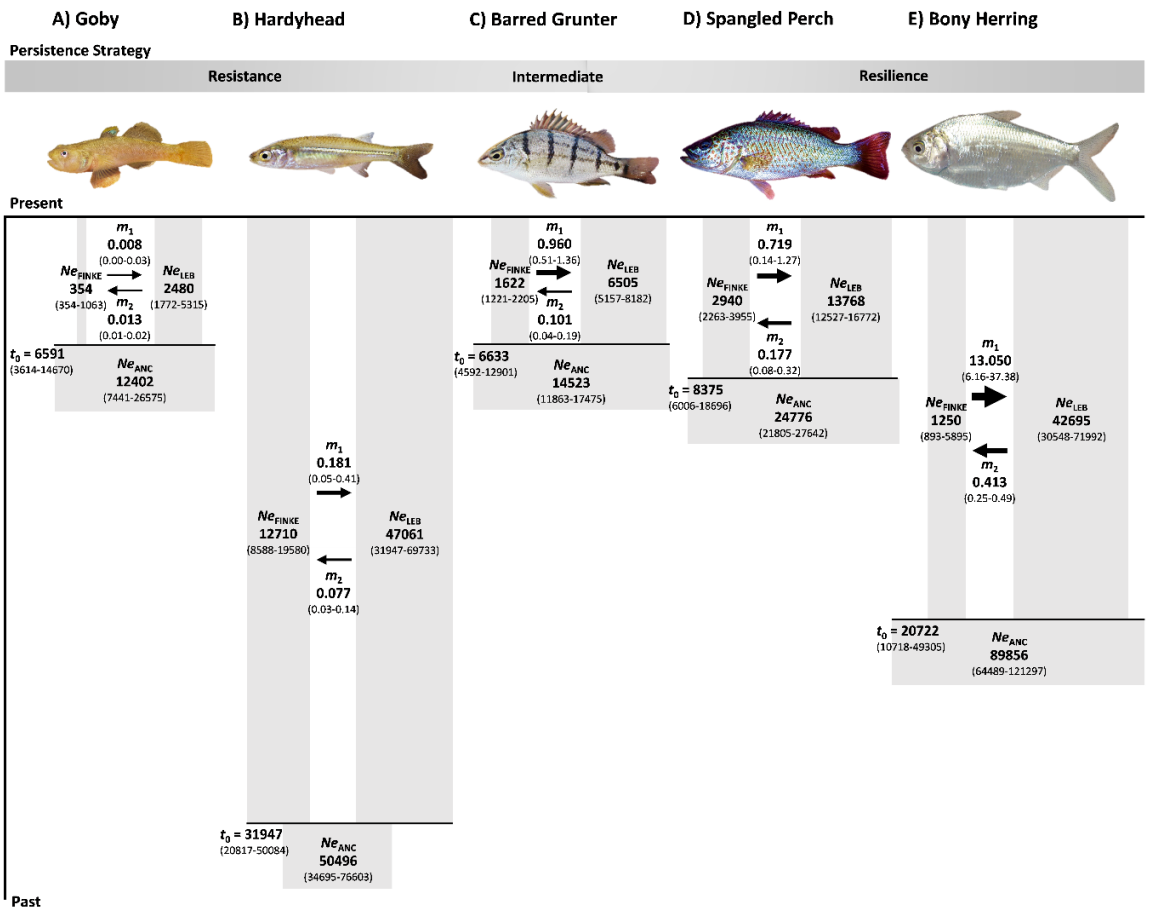


Figure 2. Schematic depiction of the two-population (Finke River versus wider Lake Eyre Basin, LEB) isolation-with migration model and parameter estimates (high point (95% confidence interval)) for five fish taxa: A) Finke goby *Chlamydogobius japalpa* and desert goby *Ch. eremius*; B) Finke hardyhead *Craterocephalus centralis* and Lake Eyre hardyhead *Cr. eyresii*; C) barred grunter *Amniataba percoides*; D) spangled perch *Leiopotherapon unicolor*; and E) bony herring *Nematalosa erebi.* In each species' model, the Finke River population is presented on the left, and the wider LEB population on the right. Parameters include divergence time (t_0, years before present), forward-in-time population immigration rates per year ($2N_em$, effective rate at which genes enter a population per year) into the LEB and Finke populations (m_1, m_2), and effective population sizes of the ancestral ($N_{e\mathrm{ANC}}$) and contemporary ($N_{e\mathrm{FINKE}}$, $N_{e\mathrm{LEB}}$) populations. Bar widths approximate population sizes (not to scale). Fish images are not to scale. Photo Credit: Michael Hammer and Ross Felix.

All taxa showed evidence of low but non-zero gene flow between the Finke River and wider LEB since population divergence (all but one estimates <1 migrant per generation; Fig. 2). The estimates of gene flow into the Finke since divergence aligned with persistence strategy: resilience-strategists had the highest levels of gene flow, followed by intermediate, with lowest estimates for resistance-strategists. A similar pattern, again following persistence strategy, was also observed for gene flow out of the Finke. For Finke, number of individuals

emigrating was higher than number immigrating for all taxa except goby (for which the estimate of Finke-to-LEB gene flow overlapped zero and gene flow in the other direction was very low).

For all taxa except the hardyhead, the estimated combined effective population sizes (N_e) of the contemporary populations (Finke and wider LEB) were much smaller than the ancestral N_e, indicating that populations in the LEB have declined over Pleistocene/Holocene timescales (Fig. 2). In contrast, the hardyhead showed little difference between ancestral and contemporary N_e, suggesting this taxon has not undergone widespread decline. Neither the contemporary nor ancestral N_e, nor the change in N_e since divergence, showed any trend with persistence strategy. While all taxa had lower N_e in the Finke than in the wider LEB, there was large variation, with N_e being 30-fold smaller in Finke goby than hardyhead (N_e = 354 vs 12,710; Fig. 2). There was great variation between species in the N_e of the Finke and LEB, with no relation to strategy: perch, grunter and hardyhead had Finke N_e that was 21–27% of LEB N_e, while goby was lower at 14%, and herring much lower, at just 3% (Fig. 2).

Genetic Diversity between Finke and Wider Lake Eyre Basin Fish Populations

All taxa exhibited significantly lower genetic diversity across Finke waterholes than across comparable samples in the wider LEB (Fig. 3; Appendix 3.5). For example, waterhole-scale samples in the Finke had allelic richness that was 34–88% less than in the wider LEB.

Table 3. Genetic diversity measures for five fish taxa sampled in the Finke River and the wider Lake Eyre Basin (LEB), including: total number of waterholes with a sample size of five or more; *n*: total number of individuals; PA: private alleles, the proportion of total species alleles unique to each sampling location; P: proportion of loci that are polymorphic.

Taxon	System	Waterholes	*n*	PA	P
Goby	Finke	6	62	11.86	15.86
	LEB	14	108	65.81	98.73
Hardyhead	Finke	6	70	19.47	61.54
	LEB	9	68	39.25	94.03
Grunter	Finke	5	44	10.90	69.90
	LEB	3	19	42.89	97.23
Perch	Finke	6	46	7.24	78.09
	LEB	10	74	46.82	99.38
Herring	Finke	4	31	6.83	66.21

	LEB	17	140	47.37	99.85

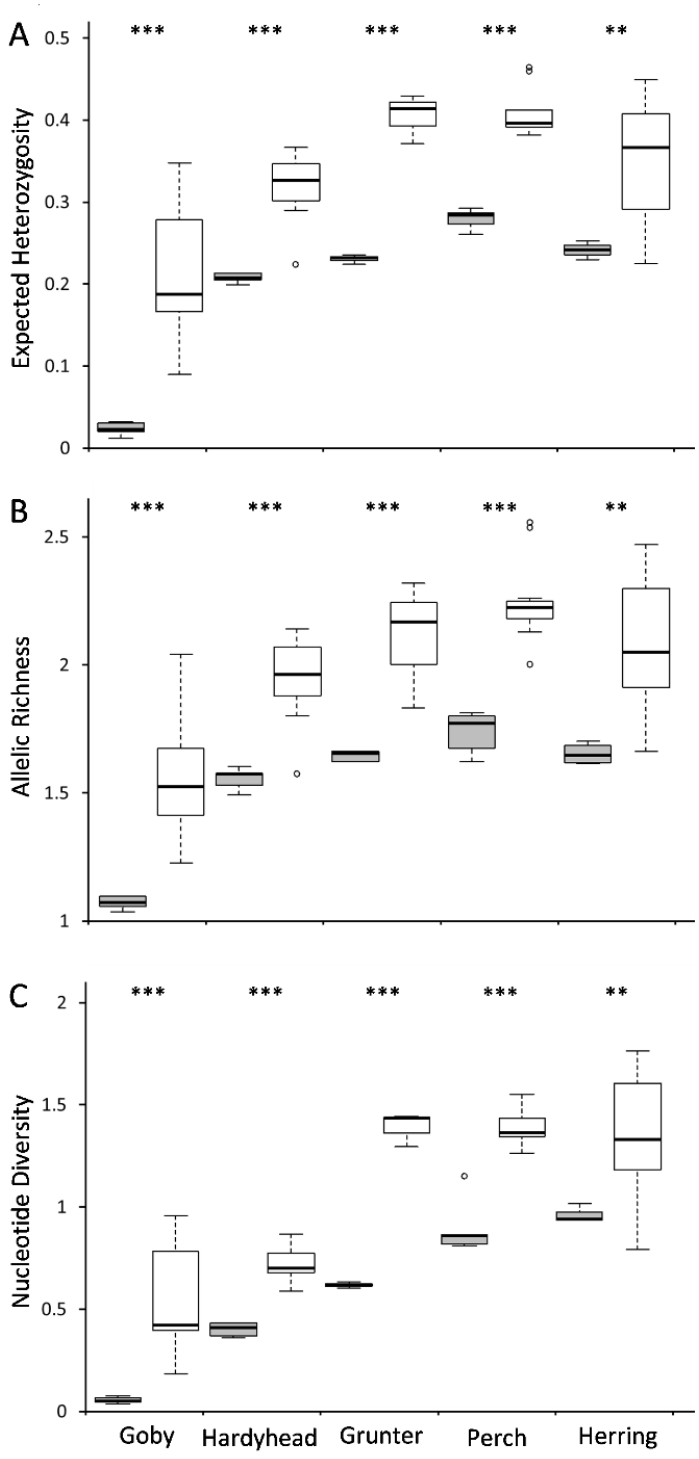


Figure 3. Genetic diversity statistics for five fish taxa averaged across sampling locations (waterholes) in the Finke River (grey) and the wider Lake Eyre Basin (white), including A) expected heterozygosity; B) mean allelic richness (adjusted for sample size of 5); and C) nucleotide diversity (x 10^{-4}). Asterisks indicate level of statistical significance between diversity statistics measured in Finke and wider LEB: * = $P < 0.05$, ** = $P < 0.01$, *** = P

< 0.001 (Appendix 3.5). Only waterholes with more than five individuals scored for genetic variation included.

All taxa showed a significant inferred effect of Finke isolation, with 95% confidence intervals excluding zero (Fig. 4). The magnitude of the inferred effect differed significantly among taxa for each diversity measure investigated: allelic richness (Q_T = 428.10, d.f. = 4, P = <0.001); expected heterozygosity (Q_T = 648.60, d.f. = 4, P = <0.001); and nucleotide diversity (Q_T = 347.67, d.f. = 4, P = <0.001). The resistant goby showed by far the greatest reductions in allelic diversity, heterozygosity and nucleotide diversity compared to wider LEB waterholes (Fig. 3). In contrast, the Finke populations of resilient perch and herring had the highest proportion of diversity compared to wider LEB waterholes. Finke goby showed extremely low levels of diversity – allelic richness of 1.07 reflects the monomorphism observed at almost 85% of 770 genome-wide loci. Almost 12% of the total goby diversity was unique to the Finke, a similar proportion to that of intermediate grunter, and higher than the two resilience-strategists. The Finke hardyhead showed a much greater level of unique diversity, with about a fifth of alleles being unique (Table 3).

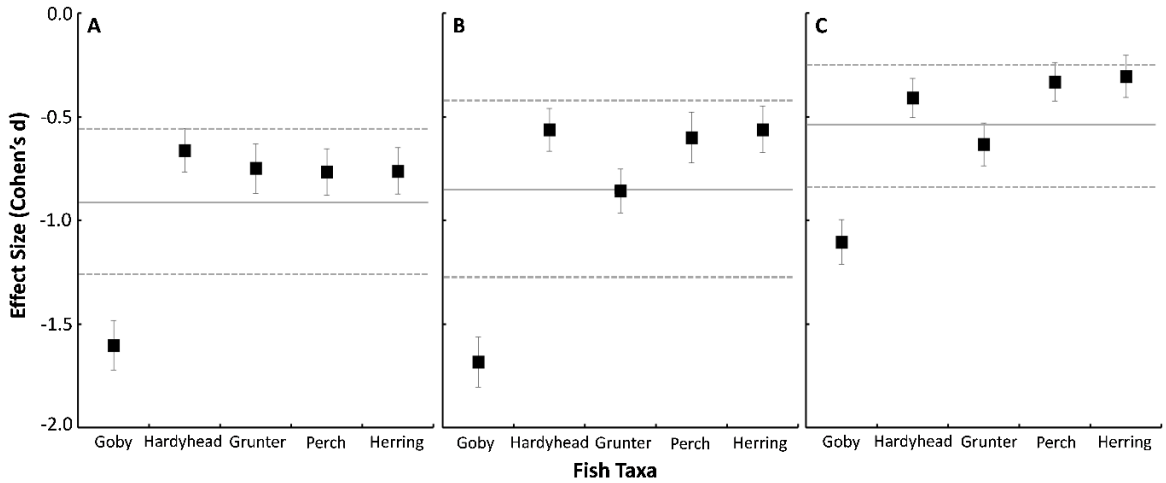


Figure 4. Effect sizes, measured as Cohen's d, of the isolation of the Finke River calculated for three genetic diversity measures: A) allelic richness; B) expected heterozygosity; and C) nucleotide diversity; for five fish taxa sampled in the Finke River and wider Lake Eyre Basin. Error bars represent the 95% confidence interval of the effect size. Cumulative weighted mean effect size and 95% confidence interval indicated by solid and dotted lines. An effect size of zero indicates no inferred effect of isolation, while negative values indicate negative effects on the genetic diversity statistic.

Discussion

We estimated the demographic history of divergence of five fish taxa following Pleistocene isolation, using a novel approach to generate a powerful genome-wide DNA sequence dataset of >700 loci for each taxon. The predictions of similar times of population divergence in the different taxa, and no gene flow in and out of the Finke River, were not met. Instead, all taxa showed low gene flow since divergence between the Finke and the wider Lake Eyre Basin (LEB). Divergence times fell into two bands, with three species diverging in the Holocene and three in the Pleistocene. Thus the data do not support rapid and complete isolation of the Finke River. Instead, the data supported a scenario of episodic disconnection, with greater levels of gene flow since divergence exhibited by resilience-strategists, and this may maintain or restore genetic variation. Below, we explore these major issues in detail, and highlight the value of estimating population parameters from coalescent analyses as an alternative to projecting from assumptions about physical landscape conditions and species' biology.

Implications of the Timing of Divergence of Finke River Fishes

Finke populations of goby, barred grunter and spangled perch are estimated to have diverged from the rest of the Lake Eyre Basin at similar times ~3–19 kya (Fig. 2). The estimate for Finke hardyhead suggests an earlier split ~20–50 kya. Bony herring are also likely to have separated early, ~10–50 kya, although the confidence intervals overlap with those of all other taxa. No species had a divergence time estimate that is clearly incompatible with the rough estimates of hydrological disconnection of the Finke River ~10–20 kya previously suggested (Kotwicki 1989; Unmack 2001b). Nonetheless, divergence time estimates for the hardyhead are earlier than (and non-overlapping with) those of the goby, grunter and perch. This implies that the disconnection of the Finke by the expansion of the Simpson Desert occurred heterogeneously in time and/or space, and the evolutionary histories of the species responded differently. The early splits seen in the hardyhead and herring coincide with a period of extreme aridity and dune-building in central Australia, from ~40 kya (Hesse & Simpson 2006; Byrne *et al.* 2008) until a time after the Last Glacial Maximum ~21 kya. The climate then became wetter again and vegetation stabilised dunes until ~11 kya when aridity returned and dune-building re-started (Luly 2001). This last phase of aridity coincides with the divergence of Finke populations of goby, grunter and perch.

These different inferred species histories could reflect quantitatively or qualitatively different responses to the same events. In addition, evidence of earlier processes might have been overwritten by later events, such as extinction and recolonisation, or homogenising gene flow. We are unable to distinguish among all possibilities, but it is notable that there was no apparent relationship between divergence time and persistence strategy. As the processes of historical divergence occurred on a different timescale to the ecological processes of persistence strategies, correlation may not be expected. Alternatively, the absence of pattern could reflect unexpected interactions of dispersal and environmental tolerance traits with environmental conditions during population divergence. In addition, stochasticity in historical population processes can be an important source of among-species differences in responses to isolating events (Leaché *et al.* 2007; Pyron & Burbrink 2010). For example, eight species-pairs of shrimp showed non-concordant divergence times following the closure of the Isthmus of Panama, despite similar dispersal mechanisms (Hurt *et al.* 2009). Human-mediated dispersal offers another possibility; while there is no direct evidence for fish translocations in the LEB by indigenous people, the region was inhabited for tens of thousands of years (Thorley 1998), and translocation of fishes is documented in other parts of Australia (e.g. Gilmore 1934, cited in Trueman 2012). Future research of fish populations in similarly isolated river systems, such as the Bulloo River that also historically connected to the wider LEB, may resolve some of this uncertainty.

Levels and Modes of Gene Flow following Divergence of Finke River Fishes

Our gene flow estimates are inconsistent with rapid and complete isolation of the Finke River. All Finke populations are estimated to have low gene flow with LEB populations since divergence, with higher levels seen in resilience-strategists, and lower rates in resistance-strategists. These coalescent migration estimates are average rates since divergence, and so could have occurred whenever gene flow was possible (Hey & Nielsen 2004). Pulses of gene flow during periods of hydrological connectivity might be envisaged for the hardyhead and herring early in their divergence when aridification was just beginning to isolate Finke populations, and perhaps again during the subsequent wetter period (Luly 2001). Finke populations of goby, grunter and perch began to diverge after this time during increasing aridity: the fact that they show subsequent non-zero gene flow suggests some connectivity, potentially for all species. Progressive aridity since then, and the lack of evidence for massive flood events connecting the Finke to the wider LEB for at least the last 850 years,

suggest declining migration rates over the last several thousand years for all taxa (Kotwicki 1986; Pickup 1991).

Modes of genetic connectivity between the Finke and the wider LEB can be further explored by considering direction of gene flow. All taxa, except goby, showed more gene flow from the Finke into the LEB than in the opposite direction. Migration into and out of the Finke since its disconnection is most likely to have occurred via the Macumba River, the proposed past and present conduit to Lake Eyre, during any sufficiently large floods, which may still occur on a millennial timescale (Pickup 1991; Duguid 2011). Migration out of the Finke is also possible in early life stages, with eggs and larvae often susceptible to downstream drift during flood events (Koehn & Crook 2013). Gene flow estimates were most asymmetrical for the herring, by far the most fecund species (33,000-880,000 eggs per spawning female; Puckridge & Walker 1990). Goby showed no gene flow out of the Finke, consistent with the lack of a pelagic larval phase, a condition unique among the studied taxa (Mossop *et al.* 2015).

The considerations above assume that expansion of the Simpson Desert was the major cause of population divergence of the sampled fishes, and that differentiation between populations of the Finke River and those of the rest of the LEB was not substantial before aridification. We consider such differentiation to be a minor contributor to estimates of gene flow on the basis of low contemporary differentiation between river systems of the LEB for many of the sampled species (Appendix 3.3). Even in the poor-dispersing goby, short-distance dispersal via small volumes of water is sufficient to connect populations over 600 km in the LEB (Mossop *et al.* 2015). In contrast, current Finke populations of all species are the most distinctive, whereas samples from the Macumba, which historically connected to the Finke, are more genetically similar to samples from distant rivers in the wider LEB than with the Finke.

Effective Population Sizes and Genetic Diversity of Finke River Fishes

All fish taxa, except hardyhead, showed a strong reduction in estimated total contemporary N_e in the Finke and wider LEB compared to ancestral N_e, consistent with loss of habitat and connectivity resulting from the aridification of central Australia (Byrne *et al.* 2008). This indicates that conditions would have been harsh during the timescale under analysis. In contrast to our prediction that resilience-strategists would show a greater decline in N_e during

isolation than resistance-strategists, there was no discernible pattern according to persistence strategy. Overall, the effect of isolation was similar for the resilience-, intermediate and one of the resistance-strategists, but much stronger for the other resistant taxon. Generally, populations with larger N_e should maintain higher levels of genetic variation and therefore have greater evolutionary potential to respond to a diverse range of future conditions (Willi *et al.* 2006; Harrisson *et al.* 2014; Wood *et al.* 2016). Due to resilience-strategists' greater reliance on connectivity for persistence, they were expected to experience reduction in N_e following isolation and so experience higher loss of genetic diversity through genetic drift. Counter to this, resilience-strategists retained relatively high levels of genetic diversity in the Finke compared to the wider LEB. This may be due to their Finke N_e being greater than 1,000 (regarded as sufficient to maintain substantial evolutionary diversity, Frankham *et al.* 2014), as well as relatively high levels of incoming gene flow offsetting loss of diversity (Keyghobadi 2007). The two resistance-strategists showed very different patterns of diversity. The Finke hardyhead showed levels of diversity approaching that of resilience-strategists and its diversity was a relatively high proportion of that in the wider LEB. In stark contrast, the Finke goby showed extreme reduction in diversity, consistent with its very low N_e and no gene flow.

Evolutionary Distinctiveness of Isolated Populations

Isolated populations of wildlife are commonly found to be genetically distinctive, including desert fishes and other aquatic taxa (e.g. Martin & Wilcox 2004; Hughes *et al.* 2009; Faulks *et al.* 2010a; Murphy *et al.* 2013). Similarly, we found the Finke populations to be the most genetically distinct in the LEB for all sampled fishes, concordant with earlier genetic and/or morphological evidence (e.g. Unmack & Dowling 2010; Bostock 2014; Mossop *et al.* 2015), and show evidence for distinctiveness of the Finke spangled perch where none was previously known (Bostock *et al.* 2006). However, while evolutionary genetic distinctiveness can flag important adaptive differences and evolutionary uniqueness, it may instead signal genetic drift, and loss of genetic diversity and evolutionary potential (Coleman *et al.* 2013; Weeks *et al.* 2016; Love Stowell *et al.* 2017) Accordingly, it is important to understand the evolutionary processes underlying empirical patterns of population differentiation. Genetic distinctiveness will accumulate more quickly with lower N_e and levels of gene flow, and with more time since divergence; because coalescent analyses can

estimate these population history parameters, they can provide important insights into the processes that drive divergences (Marko & Hart 2011).

Our application of coalescent analyses showed that the five taxa experienced very different histories of divergence between the Finke and wider LEB, largely unrelated to persistence strategy. Even the two resistance-strategists showed contrasting evolution. The Finke hardyhead diverged early, but has maintained gene flow and high N_e and levels of diversity. In contrast, the Finke goby diverged much more recently, with very low gene flow, small N_e and very low levels of genetic diversity. The goby has levels of diversity unique to the Finke approximately half that of the hardyhead. Thus, the goby would be expected to have less potential for adaptation, experiencing more genetic drift and less efficient natural selection (Frankham 2005). These different histories suggest that previously identified genetic and morphological differentiation within each taxon, while of an apparently similar nature, may have different causes and implications (Crowley & Ivantsoff 1990; Larson 1995; Unmack & Dowling 2010; Mossop *et al.* 2015). The Finke hardyhead differs from Lake Eyre hardyhead in the counts of transverse scales, fin rays and gill rakers (Crowley & Ivantsoff 1990). In the gobies, differences exist in scalation patterns, fin ray counts, and number of caudal vertebrae (Larson 1995). Such differences may reflect local adaptation, or they may be neutral or even deleterious expressions of inbreeding depression, as seen in an inbred, isolated population of another desert fish: the Sonoran topminnow (Quattro & Vrijenhoek 1989; Vrijenhoek *et al.* 1992). The low N_e (~350) in the goby is likely to preclude effective natural selection (Keller & Waller 2002; Frankham *et al.* 2014). As such, this species' morphological differences may result from inbreeding depression, genetic drift or relaxed purifying selection, rather than local adaptation. Nonetheless, it is possible for small populations to experience adaptive divergence, and stochastic events during population isolation may even promote novel adaptations (García-Ramos & Kirkpatrick 1997), as suggested for the Pecos pupfish, a desert fish native to south-western USA (Collyer *et al.* 2015). Furthermore, natural selection may be strong for characters other than those typically assessed by morphologists: desert goby populations show regional and habitat-based variation in cryptic traits relating to dispersal propensity (Moran *et al.* 2016; Mossop *et al.* 2017).

In addition to being unrelated to the ecological considerations underpinning persistence strategies, evolutionary histories were also poorly reflected by F_{ST}, commonly applied as a measure of genetic differentiation. The goby showed the greatest F_{ST} between the Finke and wider LEB, despite having the most recent divergence. The high F_{ST} seems to be driven by strong genetic drift and loss of genetic variation, rather than evolutionarily significant divergence. The other four taxa show lower F_{ST} values, despite encompassing all persistence strategies, a large range of divergence times, different migration rates, and up to an order of magnitude difference in N_e. The limitations of F_{ST} in this context are well-known, but nonetheless frequently not considered (Marko & Hart 2011).

Conservation Implications for Finke River Fishes and Other Isolated Populations

Contemporary approaches to identifying population units for conservation management emphasise the need to consider adaptive diversity (e.g. Crandall *et al.* 2000). The Finke River populations of all taxa studied here have quantified, distinct evolutionary histories involving restricted immigration over thousands of years, and substantial genetic divergence. Despite being able to provide this context and suggest relative evolutionary potential among taxa, the data do not assess the level of local adaptation of the Finke populations. Morphological characters define the Finke populations of both resistance-strategists as species, but the adaptive significance of this differentiation is unknown, and is particularly unclear for the Finke goby. Understanding local adaptation in Finke fishes could be achieved by ecological experimentation, usefully supplemented by deep-coverage genome-wide analyses of genetic variation (Pavlova *et al.* 2017).

Loss of evolutionary potential is of conservation concern in many populations with isolated distributions (Frankham *et al.* 2014). In North America, desert fishes with poorer dispersal abilities (generally resistance-strategists) are at greater extinction risk from fragmentation (Fagan 2002; Olden *et al.* 2008), and in Australia, the extreme 'Millennium Drought' (2001–2009) led to widespread fragmentation, declines and an increase in conservation listings for resistant fishes, but not resilience-strategists (McNeil *et al.* 2011a; Hammer *et al.* 2015). This pattern was not as clear-cut in our sample of Finke fishes: the resistant Finke goby has the least evolutionary potential, whereas the other resistance-strategist, hardyhead, maintained diversity and N_e. The resilient and intermediate taxa fared similarly under isolation, potentially due to the tempering effects of gene flow. We conclude that both

persistence strategies should be able to retain genetic diversity, when some gene flow is present.

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Chapter Seven

General Discussion

Within this thesis, I aimed to investigate the role of population connectivity in persistence of populations in highly fragmented environments. To do so, I applied a comparative framework and a population genomics approach to explore contemporary and historical genetic patterns and evolutionary processes in a suite of desert fishes in the Lake Eyre Basin in arid central Australia. The framework provided a way to test hypotheses about the role of population connectivity in contemporary and future population persistence. The work in this thesis addresses major knowledge gaps in our understanding of the persistence of populations (Mari et al. 2014). It also informs the conservation and management of populations in fragmented environments. Future climate change and other anthropogenic impacts are expected to result in greater habitat fragmentation globally (Woodward et al. 2010; Jaeger et al. 2014). Here, I synthesise the different aspects of my study findings and consider their wider implications for our understanding of persistence through population connectivity, future research and management

Persistence through Population Connectivity

My research has addressed key knowledge gaps in our understanding of species persistence, by focusing on the drivers of population connectivity: structural connectivity (including hydrological connectivity) and species' ecology. These drivers were found to be important for contemporary and future persistence of fishes in the LEB, and have implications for other aquatic taxa within this system and other desert freshwaters worldwide.

How does Structural Connectivity influence Contemporary Persistence?

I determined that population connectivity of aquatic taxa in desert freshwaters is highly dependent on several aspects of structural connectivity, which describes how movement of individuals is facilitated by the environment (Chapter Two). Population connectivity decreased at larger scales for aquatic fauna in desert freshwaters globally, with significantly greater connectivity at the within-river scale than among-rivers. This pattern was also found

to hold true for fishes in the LEB (Chapter Five). Distance itself is also important, with fishes found to exhibit significant Isolation By Distance within and among rivers of the LEB. These patterns often reflected the dendritic patterns of rivers and the wider Basin, showing that population connectivity is also influenced by the spatial hierarchy of river systems (Meffe & Vrijenhoek 1988). This is surprising, given that high extinction and recolonisation rates in highly fragmented systems, between populations from distant sites, are expected to erase much of the genetic structure of populations. While this result may be driven by lower extinction and recolonisation rates, it could result from the greater power to detect structure provided by a genomics approach over traditional genetic markers.

In the LEB and other desert freshwaters, local environmental factors influence structural connectivity, including the number and location of habitats, and the frequency and variability of connecting flows (i.e. hydrological connectivity; Hughes et al. 2013). Accordingly, it was expected that rivers with different flow regimes or channel morphology would exhibit differences in structural, and therefore population, connectivity. However, population connectivity of all species was largely consistent and relatively high within each LEB river, suggesting that these differences were not a major influence. Further investigation of other within-river environmental variables, including differences between permanent and temporary waterholes (evolutionary and ecological refuges; Davis et al. 2013) was beyond the scope of this thesis, but would provide greater insight into fine-scale drivers of connectivity and persistence. At larger scales, structural connectivity amongst rivers appears to influence fish populations and communities within the LEB (see Chapters Four, Five, and Six). Rivers that experience the lowest flooding frequencies, resulting in fewer hydrological connections with other river systems, exhibit the lowest population connectivity. Consequently, recolonisation of these rivers following a hypothetical extirpation of fish populations is less likely than for rivers with greater hydrological connectivity. Variables other than hydrology may be important drivers of structural connectivity too, with an unknown barrier restricting population connectivity between Cooper Creek and other LEB rivers. Identifying and understanding the drivers of structural connectivity, including whether they are natural or anthropogenic, is important for management of population connectivity within desert freshwaters.

How does Species' Ecology influence Contemporary Persistence?

I performed an extensive literature review and meta-analysis that found species' ecology, especially dispersal ability, to be a key driver of population connectivity in desert freshwaters globally (Murphy et al. 2015). Despite this, many studies reviewed in Chapter Two did not explicitly consider species' ecology, and may have missed an opportunity to understand the processes underlying population connectivity. In Chapters Five and Six, I explicitly considered and compared the influence of the ecology of five diverse fishes on their population connectivity. Rather than considering a single trait (such as dispersal ability), a more holistic approach was taken. This required synthesising species' ecology into persistence strategies, incorporating dispersal ability and environmental tolerance. While this limits our understanding of the individual influences of particular traits, it does prevent conflation of the effects of one trait with others. Accordingly, persistence strategies, or similarly inclusive descriptions of species' ecology, provide an opportunity to more fully understand population connectivity and predict species responses to fragmented environments. Such strategies are also utilised by a range of other aquatic fauna in deserts worldwide (Bogan et al. 2017), and the general conclusions reached here are likely to be applicable to species with similar strategies.

Within and among the rivers of the LEB, persistence strategies (ranging from resistance to resilience) were found to influence population connectivity. Resilience-strategists showed high population connectivity, as expected for taxa that rely on recolonisation for contemporary persistence. The two resistance-strategists were expected to show low population connectivity, but instead exhibited contrasting levels: one low and one not low. The latter result may arise from incorrect assignment of strategy, or ecologies that have yet to be understood. Resistance and resilience traits are not mutually exclusive, and some species possess and utilise both (e.g. Chester et al. 2015), potentially leading to high population connectivity. While the cause of the unexpectedly high population connectivity detected for one resilient taxon here is not clear, it demonstrates that insights provided by genetic data can reveal patterns and processes not easily understood from observational research alone. Contemporary persistence is clearly facilitated by both strategies, although the smaller numbers of resistant taxa within the LEB and other desert freshwaters suggest that resilience may be a more effective strategy for desert fishes.

How does Structural Connectivity influence Future Persistence?

I have shown that a loss of structural connectivity between rivers reduced the genetic diversity and evolutionary potential of fish populations, likely compromising their future persistence (Chapter Six). The loss of diversity detected in Finke River fish populations is driven by the loss of gene flow from other systems, i.e. lower genetic connectivity, which increases genetic drift and other negative genetic processes within rivers (Keyghobadi 2007; Frankham et al. 2017). In is unclear whether time since isolation impacts maintenance of genetic diversity, but negative genetic effects may accumulate temporally. To gain insights into longer-term impacts of a loss of structural connectivity on desert fish populations, future research could investigate the fish populations in the Bulloo River. This river system was previously hydrologically linked to the wider LEB, but was likely disconnected much earlier than the Finke, so its fish populations are expected to have been isolated for far longer. The resistant taxa studied here are absent from the Bulloo, while the resilient taxa are still present, potentially suggesting that future persistence in isolation is less likely for the former.

Within this thesis, the population connectivity within and among river systems has been investigated, but research at additional scales may also provide insights into future persistence. The entire LEB lacks contemporary structural connectivity to other basins, and has likely been isolated for tens of thousands of years (Wager & Unmack 2000). The LEB is expected to have much lower structural connectivity than adjacent basins, including the mesic Murray-Darling Basin and the tropical basins to the north (Chapter Three). Understanding how these differences between basins have impacted fish populations (including of species shared among these basins) would provide insights into potential future impacts in mesic and tropical basins under climate change scenarios. At smaller scales, investigation of small isolated populations within the LEB, such as fishes restricted to remote springs, should also provide useful insights. In extreme cases, riverine waterholes in the LEB may become isolated, for example through anthropogenic construction of barriers (such as dams). Comparisons of population connectivity of fishes at these very broad and very fine scales would provide further insights into the effects of reduced hydrological connectivity on fish populations.

How does Species' Ecology influence Future Persistence?

I have demonstrated that population connectivity, and likely future persistence, differs among species with different traits (Chapters Two, Five, Six). Within desert freshwaters, resilient and resistant taxa are expected to lose genetic diversity over time, the former through repeated bottlenecks resulting from high subpopulation extinction rates, and the latter from subpopulation isolation leading to inbreeding and genetic drift (Frankham et al. 1999; Frankham et al. 2017). Accordingly, while desert fishes exhibit a range of different persistence strategies, they all appear to be at risk of losing genetic diversity, and therefore evolutionary potential (Harrisson et al. 2014). Within this thesis, I was unable to directly compare levels of genetic diversity among taxa as different loci were used for each species. Future research using comparable markers among species would allow greater exploration of the effects of these strategies on evolutionary potential of desert fishes.

However, I have demonstrated that populations of a species (or sister species pair) within isolated river systems have lower diversity than those in connected rivers, and that the degree of reduction in diversity differs among taxa (Chapter Six). While these differences were not completely linked to persistence strategy, species that maintained population connectivity with populations in other rivers during the isolation process retained greater diversity. Accordingly, species with strategies and other ecological traits that enable population connectivity are likely to be better placed to persist in the face of environmental change. Further anthropogenic impacts are likely to negatively affect all desert fishes, but resistant taxa with weak dispersal ability are expected to be most vulnerable, especially where individual habitats are threatened. Overall, population connectivity is vital for the future persistence of desert fishes in the LEB and other desert freshwaters, and management should seek to maintain population connectivity to ensure population persistence.

Methodological Approaches for Future Research

The approach of applying a comparative framework and genomics to a population-level study of ecology and evolution is still relatively novel, and this study provides the opportunity to evaluate its utility and efficiency. The use of comparative frameworks is not new, but has become increasingly common in ecological studies, especially for determining drivers of processes (Andrew et al. 2013). Here, this framework enabled detection of patterns shared among taxa (driven by structural connectivity) and patterns that differed among taxa (driven by species' ecology). Neither would have been identifiable in single-species studies, greatly limiting the inferences that could be made regarding general processes. Comparative frameworks are efficient for developing general principles for scientific understanding and conservation management, and should be implemented where practical (Pauls et al. 2014; Mims et al. 2017).

Genomic approaches make comparative studies more tractable as they provide greater statistical power with which to investigate ecological and evolutionary patterns and processes (Ellegren 2014). Here, I utilised a large (hundreds of loci per taxon) genomic dataset in two distinct ways. While biallelic SNP datasets are frequently generated from genomic data, here all variable sites were extracted from each locus, resulting in a multi-allelic genotype dataset that captures all sequence variation. This approach differs from most genomic studies that utilise only the first SNP of each locus (e.g. typical RAD-seq studies), and therefore ignore much of the variation in their datasets. This dataset provided far deeper insights than traditional markers have done and revealed previously undetected genetic patterns. For example, previous genetic studies of spangled perch across Australia using allozymes and mitochondrial DNA did not detect any population structure in the range of this widespread fish (Bostock et al. 2006). In fact, it was even suggested that further population genetic study of this species would not be beneficial given its minimal divergence and structure across an entire continent (Bostock 2014). However, I detected significant genetic structure among river-scale populations of this species within the LEB with population genomics. While genotype data are useful for understanding and exploring genetic patterns and some contemporary processes, they are not useful for elucidating historical evolutionary processes. To address this, the genomic dataset was used in sequence format for exploration of demographic history with coalescent theory, another approach that is also limited with traditional markers (Kuhner 2009). Inclusion of genotype and sequence

data in population genomics offers a powerful approach to explore contemporary and historical genetic patterns and evolutionary processes.

While genomic data provide greater insights than traditional genetic markers, they also present a number of challenges for researchers (Jones & Good 2016). Because many resources utilised for population genetics are unavailable for population genomics, performing even basic analyses may require substantial formatting and bioinformatics. Making full use of the data is also difficult, and often requires significant computing power, which may be inaccessible to many researchers. The large datasets often exceed the capacity of many genetic analysis programs, restricting their utility for population genomics. As many programs were developed for one or few traditional markers, use of hundreds is often prohibitively slow at best. Overall, however, the benefits of population genomics outweigh its costs, and challenges will diminish as more researchers utilise this powerful tool, and computing technologies improve.

Within this thesis, I utilised a novel high-throughput targeted sequence capture approach ('MetaPrep') that greatly increases cost-effectiveness by allowing samples of different taxa to be pooled together for sequencing. Because this technique sequences samples of diverse taxa simultaneously, and costs per sample are reduced as additional species are added, it is highly suited to comparative framework approaches. However, this method does present substantial challenges for researchers. The 'anonymous but unique' nature of the loci chosen means that they are not necessarily representative of the whole genome. Consequently, it is extremely difficult to provide an estimate of the mutation rate of the sequenced loci. Until these rates can be reliably estimated, researchers must either incorporate loci of known mutation rate (e.g. mitochondrial DNA markers), or use very wide estimates of mutation rates when performing analyses that require mutation rates. Further, loci selected by MetaPrep (and their mutation rates) are not necessarily comparable among species. Where more closely-related taxa are included (such as the two Terapontidae in this study), MetaPrep should tend to select faster-evolving loci that are not shared among relatives. In contrast, where taxa are not closely-related, MetaPrep is unlikely to select solely fast-evolving loci. Therefore, using these data in a comparative framework is challenging; for example, measures of genetic diversity cannot be directly compared among species. However, as demonstrated in this thesis, these challenges are not insurmountable.

Management for Population Persistence

The threats to population persistence in fragmented environments have been highlighted throughout this thesis. The recommendations for conservation management that have been made are expanded upon here. Habitat fragmentation across many biomes is likely to increase via climate change and other anthropogenic impacts (Opdam & Wascher 2004; Bellard et al. 2012). In desert freshwaters, the decrease in structural connectivity will create additional challenges for the future persistence of populations (Woodward et al. 2010; Jaeger et al. 2014). Given that population connectivity is vital for population persistence, management should aim to maintain population connectivity wherever possible.

While management based on population connectivity is an effective approach, determining population connectivity can be difficult, especially when resources are limited. Identification of general relationships between species' ecology or structural connectivity and population connectivity can facilitate management of groups of taxa, without requiring species-specific research (Hughes et al. 2013; Mims et al. 2017). Using a comparative framework, as undertaken here, is an effective approach for such research, especially where a range of species exhibiting different traits within a single landscape are investigated (Andrew et al. 2013). Such studies are now becoming more common, and as more begin to use population genomic tools, the inferences made are likely to provide greater insights into the population processes that contribute to population connectivity (Pauls et al. 2014).

Communicating, and ideally collaborating, with biodiversity managers is imperative for implementing effective, evidence-based conservation actions (Toomey et al. 2017). Providing standardised conclusions across a range of taxa is one way in which clear messages can be communicated. Population connectivity models offer a useful way of summarising species' population connectivity for managers (Hughes et al. 2013). A number of models apply directly to desert freshwaters, including the Stream Hierarchy and Death Valley Models developed specifically for aquatic taxa in these fragmented systems (Meffe & Vrijenhoek 1988). These models have implications for management, for example species following a panmictic model will require greater preservation of hydrological connectivity than those species following the Death Valley model (discussed further in Hughes et al. 2013; Murphy et al. 2015).

While simple, these models provide a useful starting point for researchers and managers, and can be extended as required based on available knowledge. Differences in species' ecology, for example, resulted in LEB fishes exhibiting different degrees of structure within-rivers, although all followed the Stream Hierarchy Model (Chapter Five). They are also threatened by different processes, with resistant taxa likely to be more affected by loss of refugial habitats, and resilient taxa more affected by a loss of connecting flows between habitats (Davis et al. 2013; Bogan et al. 2017). However, a major conclusion from this study is that persistence strategy alone is not necessarily a good predictor of population connectivity. While strategies (based on field observations) correctly predicted population connectivity for four out of five fishes studied, additional study of population genomics was required to confirm this and provided substantial detail that could not be gained in any other way. Accordingly, where possible (and especially for threatened taxa) researchers should include population genomics in management plans (as discussed above, application of this approach is rapidly becoming viable for conservation).

Connectivity models can also be extended by incorporating local structural connectivity. For example, within the LEB there were clear differences in structural connectivity among rivers. Again, these differences were generally not apparent from observational studies and elucidated via genetic analyses. The distinct populations noted in several river systems are attributed to reduced hydrological connectivity, but the effects of this reduction differ among rivers and taxa (Chapter Five). Even among some hydrologically-connected rivers there was low population connectivity, suggesting that not all structural connectivity is easily predicted in desert freshwaters, and may not reflect contemporary processes.

Contemporary approaches to conservation management emphasise the need to consider adaptive diversity and evolutionary potential (Crandall et al. 2000; Harrisson et al. 2014). Genetically-distinct populations are often afforded substantial management resources, but may lack unique diversity or the capacity to evolve in response to environmental change (Coleman et al. 2013). Within the LEB, fish populations in the Finke River were found to be distinct, but at least one appears to be highly inbred and with extremely little unique diversity. Maintaining this population in isolation may not lead to the best conservation outcomes, with assisted gene flow a potential option to restore some diversity and evolutionary potential (Harrisson et al. 2016). Within the wider LEB, future declines in population

connectivity among habitats may also lead to loss of diversity and other negative genetic effects. In such scenarios, assisted gene flow may also be a viable way of maintaining natural population connectivity (Pavlova et al. 2017). While such interventions have rarely been implemented among genetically-distinct populations, especially those isolated by historic, natural processes, they are expected to lead to positive outcomes for future population persistence (Frankham et al. 2017).

Conclusions

This thesis has contributed new knowledge in three key areas. Firstly, it demonstrates the utility, efficiency and power of combining a comparative framework with population genomics to study multiple species and reveal general patterns. While challenging, such an approach provides insights that cannot be otherwise gained, and is recommended for future studies of ecological and evolutionary processes. Secondly, this study elucidates the contemporary population genetics and evolutionary history of a suite of desert fishes of the Lake Eyre Basin in central Australia. These species inhabit one of the most extreme and fragmented environments on the planet, and their persistence is unexpected and impressive. The new knowledge gained for each of the study species is useful for understanding their population histories, explaining contemporary population processes, and predicting their futures. Thirdly, this study enhances our understanding of the roles of structural connectivity and species' ecology in driving population connectivity in highly fragmented environments. This understanding enables predictions to be made regarding the contemporary and future persistence of populations of different species in different locations. These predictions can be used to inform conservation management and to direct resources towards the most effective actions. Overall, the work included in this thesis contributes substantial new information and understanding that can benefit researchers and biodiversity managers.

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Appendix One

Supporting Information for Chapter Two

Appendix 1.1 – Dataset of Studies of Desert Freshwater Connectivity

Table detailing the dataset of 133 desert freshwater connectivity studies reviewed to give an overview of the trends in the field, with selected details of study systems, methodologies and conclusions noted. A full reference list of sources is provided below.

Table 1. Dataset of 133 desert freshwater connectivity studies reviewed to give an overview of the trends in the field, with selected details of study systems, methodologies and conclusions noted. Details include: Species – species names as provided in source; Class – taxonomic class of organism; Disp – predicted dispersal ability of organism (low, moderate, high – see Methods for definitions); Study Location – country where species sampled (number only where more than two countries sampled); Habitat Type – habitat type sampled (pools, rivers, springs or multiple); Markers Used – class/es of molecular marker used in study (allozymes (Allo), amplified fragment length polymorphisms (AFLP), nuclear DNA sequences (nDNA), microsatellites (msats), mitochondrial DNA (mtDNA), randomly amplified polymorphic DNA (RAPD), restricted fragment length polymorphisms (RFLP) and single-primer amplification reaction (SPAR)); Analytical Method – method used for analyses of genetic data (deterministic or probabilistic*); Connectivity Model Concluded – conclusion of connectivity model or description of gene flow (Panmixia, Isolation By Distance (IBD), Stream Hierarchy Model (SHM), Death Valley Model (DVM), no, restricted or high gene flow (GF)), at each of three scales*. *See Methods section for definitions of these terms.

								Connectivity Model Concluded		
Source	**Species**	**Class**	**Disp**	**Study Location**	**Habitat Type**	**Markers Used**	**Analytical Method**	**Within Systems**	**Between Systems**	**Between Basins**
Echelle *et al.* 1987	*Cyprinodon bovinus*	Osteichthyes	Low	USA	Multiple	Allo	Deterministic	High GF	–	–
	Cyprinodon elegans	Osteichthyes	Low	USA	Springs	Allo	Deterministic	IBD	–	–
	Cyprinodon pecosensis	Osteichthyes	Low	USA	Rivers	Allo	Deterministic	High GF	–	–
	Cyprinodon tularosa	Osteichthyes	Low	USA	Springs	Allo	Deterministic	Low GF	–	–
Echelle *et al.* 1989	*Gambusia nobilis*	Osteichthyes	Mod	USA	Springs	Allo	Deterministic	High GF	Restricted GF	–
Ponder *et al.* 1995	*Fonscochlea accepta*	Gastropoda	Mod	Australia	Springs	Allo	Deterministic	IBD	–	–
	Fonscochlea aquatica	Gastropoda	Mod	Australia	Springs	Allo	Deterministic	IBD	–	–
	Fonscochlea variabilis	Gastropoda	Mod	Australia	Springs	Allo	Deterministic	IBD	–	–
	Fonscochlea zeidleri	Gastropoda	Mod	Australia	Springs	Allo	Deterministic	IBD	–	–
	Trochidrobia punicea	Gastropoda	Low	Australia	Springs	Allo	Deterministic	IBD	–	–
	Trochidrobia smithi	Gastropoda	Low	Australia	Springs	Allo	Deterministic	IBD	–	–
Quattro *et al.* 1996	*Poeciliopsis occidentalis*	Osteichthyes	Mod	USA	Rivers	mtDNA	Deterministic	High GF	Low GF	Low GF
Tibbets & Dowling 1996	*Tiaroga cobitis*	Osteichthyes	Mod	USA	Rivers	Allo, RFLP	Deterministic	No GF	No GF	–
	Meda fulgida	Osteichthyes	Mod	USA	Rivers	Allo, RFLP	Deterministic	Restricted GF	No GF	–
	Agosia chrysogaster	Osteichthyes	High	USA	Rivers	Allo, RFLP	Deterministic	Restricted GF	No GF	–
Davies *et al.* 1997	*Branchinecta sandiegonensis*	Branchiopoda	Low	USA	Pools	Allo	Deterministic	–	Low GF	–
Thomas *et al.* 1997	*Hyalella azteca*	Malacostraca	Low	USA	Pools	RAPD	Deterministic	Restricted GF	–	–
	Hyalella montezuma	Malacostraca	Low	USA	Pools	RAPD	Deterministic	IBD	–	–
Viard *et al.* 1997	*Bulinus truncatu*	Gastropoda	Low	8 Countries	Rivers	Msats	Deterministic	IBD	Restricted GF	No GF
Duvernell & Turner 1998	*Cyprinodon nevadensis*	Osteichthyes	Low	USA	Rivers	mtDNA	Deterministic	Restricted GF	No GF	–

Source	Species	Class	Disp	Study Location	Habitat Type	Markers Used	Analytical Method	Connectivity Model Concluded		
								Within Systems	Between Systems	Between Basins
	Cyprinodon salinus	Osteichthyes	Low	USA	Rivers	mtDNA	Deterministic	No GF	–	–
Johnson & Jordan 2000	*Gila copei*	Osteichthyes	Mod	USA	Rivers	mtDNA	Deterministic	–	–	No GF
Miller *et al.* 2000	*Oxylema haydeni*	Gastropoda	Low	USA	Springs	AFLP, mtDNA	Deterministic	No GF	–	–
Mesquita *et al.* 2001	*Chondrostoma lusitanicum*	Osteichthyes	Mod	Portugal	Rivers	mtDNA, RFLP	Deterministic	High GF	–	Low GF
Cook *et al.* 2002	*Macrobrachium australiense*	Malacostraca	Mod	Australia	Rivers	Allo, mtDNA	Deterministic	Panmixia	DVM	DVM
Johnson, 2002	*Gila atraria*	Osteichthyes	Mod	USA	Rivers	mtDNA	Deterministic	–	IBD	No GF
Miller *et al.* 2002	*Ambrysus thermarum*	Insecta	Low	USA	Rivers	AFLP	Deterministic	High GF	High GF	–
	Psephenus montanus	Insecta	Low	USA	Rivers	AFLP	Deterministic	No GF	No GF	–
Nielsen & Sage 2002	*Oncorhynchus clarki*	Osteichthyes	Mod	USA	Rivers	Msats	Deterministic	–	No GF	–
Douglas *et al.* 2003	*Catostomus latipinnis*	Osteichthyes	Mod	USA	Rivers	mtDNA	Probabilistic	High GF	High GF	–
Hughes & Hillyer 2003	*Cherax destructor*	Malacostraca	Mod	Australia	Rivers	mtDNA	Deterministic	High GF	Low GF	Low GF
Whitehead *et al.* 2003	*Catostomus occidentalis*	Osteichthyes	Mod	USA	Rivers	AFLP, Msats	Deterministic	IBD	Restricted GF	–
Carini & Hughes 2004	*Macrobrachium australiense*	Malacostraca	Mod	Australia	Rivers	mtDNA	Probabilistic	Restricted GF	Restricted GF	No GF
Gervasio *et al.* 2004	*Gammarus pecos*	Malacostraca	Low	USA	Springs	Allo	Deterministic	IBD	–	–
Gow *et al.* 2004	*Bulinus forskalii*	Gastropoda	Low	Cameroon	Multiple	Msats	Probabilistic	–	High GF	–
Hughes *et al.* 2004	*Velesunio* spp. A	Bivalvia	Mod	Australia	Rivers	Allo, mtDNA	Probabilistic	Restricted GF	No GF	–
	Velesunio spp. B	Bivalvia	Mod	Australia	Rivers	Allo, mtDNA	Probabilistic	Restricted GF	–	–
	Velesunio spp. C	Bivalvia	Mod	Australia	Rivers	Allo, mtDNA	Probabilistic	Restricted GF	Low GF	No GF
	Velesunio spp. D	Bivalvia	Mod	Australia	Rivers	Allo, mtDNA	Probabilistic	Restricted GF	–	–
Martin & Wilcox 2004	*Cyprinodon nevadensis*	Osteichthyes	Low	USA	Springs	Msats	Deterministic	Restricted GF	Low GF	–
Mock *et al.* 2004	*Anodonta californiensis*	Bivalvia	Mod	USA	Multiple	AFLP, mtDNA	Deterministic	–	No GF	–
Moline *et al.* 2004	*Nymphophilus minckleyi*	Gastropoda	Low	Mexico	Springs	Allo	Deterministic	High GF	–	–
Murphy & Austin 2004	*Macrobrachium australiense*	Malacostraca	Mod	Australia	Rivers	mtDNA	Deterministic	–	Restricted GF	No GF
Nguyen *et al.* 2004	*Cherax destructor*	Malacostraca	Mod	Australia	Rivers	mtDNA	Deterministic	Restricted GF	Low GF	No GF
Alo & Turner 2005	*Hybognathus amarus*	Osteichthyes	Mod	USA	Rivers	Msats, mtDNA	Deterministic	High GF	–	–
Hershler *et al.* 2005	*Tryonia porrecta*	Gastropoda	Low	USA	Springs	Allo, mtDNA	Deterministic	–	–	Restricted GF
Johnson 2005	*Mexipyrgus churinceanus*	Gastropoda	Low	Mexico	Multiple	mtDNA	Probabilistic	IBD	No GF	–
Mamuris *et al.* 2005	*Ladigesocypris ghigii*	Osteichthyes	Mod	Greece	Rivers	RAPD, RFLP	Deterministic	High GF	–	No GF
Mesquita *et al.* 2005	*Squalius aradensis*	Osteichthyes	Mod	Portugal	Rivers	Msats, mtDNA	Deterministic	–	Restricted GF	No GF
Mock & Miller 2005	*Iotichthys phlegethontis*	Osteichthyes	Low	USA	Springs	AFLP, mtDNA	Deterministic	Restricted GF	No GF	–
Bostock *et al.* 2006	*Leiopotherapon unicolor*	Osteichthyes	High	Australia	Rivers	Allo, mtDNA	Deterministic	High GF	High GF	High GF
Carini & Hughes 2006	*Notopala sublineata*	Gastropoda	Low	Australia	Rivers	Allo, mtDNA	Probabilistic	Restricted GF	Restricted GF	No GF

Source	Species	Class	Disp	Study Location	Habitat Type	Markers Used	Analytical Method	Connectivity Model Concluded		
								Within Systems	Between Systems	Between Basins
Carini et al 2006	*Macrobrachium australiense*	Malacostraca	Mod	Australia	Rivers	mtDNA	Deterministic	High GF	–	–
	Notopala sublineata	Gastropoda	Low	Australia	Rivers	Allo, mtDNA	Deterministic	High GF	–	–
Carson & Dowling 2006	*Cyprinodon atrorus*	Osteichthyes	Low	Mexico	Multiple	nDNA, mtDNA	Deterministic	Restricted GF	–	No GF
	Cyprinodon bifasciatus	Osteichthyes	Low	Mexico	Multiple	nDNA, mtDNA	Deterministic	Restricted GF	–	No GF
Cegelski *et al.* 2006	*Oncorhynchus clarkii*	Osteichthyes	Mod	USA	Rivers	Msats	Probabilistic	Multiple	Restricted GF	–
Colgan *et al.* 2006	*Caldicochlea globosa*	Gastropoda	Low	Australia	Springs	mtDNA	Deterministic	No GF	–	–
	Caldicochlea harrisi	Gastropoda	Low	Australia	Springs	mtDNA	Deterministic	No GF	–	–
Huey *et al.* 2006	*Neosilurus hyrtlii*	Osteichthyes	Mod	Australia	Rivers	Allo, mtDNA, Msats	Probabilistic	Panmixia	No GF	No GF
	Porochilus argenteus	Osteichthyes	Mod	Australia	Rivers	mtDNA, Msats	Probabilistic	Panmixia	–	–
Hughes & Hillyer 2006	*Nematolosa erebi*	Osteichthyes	High	Australia	Rivers	Allo, mtDNA	Probabilistic	Restricted GF	No GF	No GF
	Retropinna semoni	Osteichthyes	Low	Australia	Rivers	Allo, mtDNA	Probabilistic	SHM	No GF	No GF
Miller *et al.* 2006	*Valvata utahensis*	Gastropoda	Low	USA	Rivers	AFLP, mtDNA	Deterministic	High GF	IBD	–
Mock *et al.* 2006	*Catostomus ardens*	Osteichthyes	Mod	USA	Rivers	mtDNA, AFLP	Deterministic	–	Restricted GF	No GF
Bernardi *et al.* 2007	*Fundulus lima*	Osteichthyes	Low	Mexico	Springs	mtDNA	Probabilistic	–	No GF	–
Hulsmans *et al.* 2007	*Branchipodopsis wolfi*	Branchiopoda	Mod	Botswana	Pools	Allo	Deterministic	IBD	IBD	–
Zickovich & Bohonak 2007	*Hyalella azteca*	Malacostraca	Low	USA	Rivers	mtDNA	Probabilistic	IBD	–	–
Huey *et al*. 2008	*Neosilurus hyrtlii*	Osteichthyes	Mod	Australia	Rivers	Allo, mtDNA, msats	Probabilistic	SHM	SHM	No GF
Masci *et al*. 2008	*Nematalosa erebi*	Osteichthyes	High	Australia	Rivers	mtDNA	Probabilistic	IBD	IBD	No GF
	Macrobrachium australiense	Malacostraca	Mod	Australia	Rivers	mtDNA	Probabilistic	–	–	No GF
Munoz *et al.* 2008	*Artemia salina*	Anacostraca	High	8 Countries	Pools	mtDNA	Deterministic	–	–	No GF
Pamponet *et al.* 2008	*Astyanax* aff. *bimaculatus*	Osteichthyes	Mod	Brazil	Rivers	RAPD, SPAR	Deterministic	–	No GF	No GF
Sousa *et al.* 2008	*Chondrostoma lusitanicum*	Osteichthyes	Mod	Portugal	Rivers	mtDNA, Msats	Deterministic	–	Restricted GF	No GF
Worthington Wilmer *et al.* 2008	*Fonscochlea accepta*	Gastropoda	Mod	Australia	Springs	Msats	Probabilistic	High GF	IBD	–
Loftis *et al.* 2009	*Cyprinodon eremus*	Osteichthyes	Low	Mexico, USA	Rivers	Msats	Deterministic	IBD	–	–
Murphy *et al.* 2009	*Austrochiltonia dalhousiensis*	Malacostraca	Low	Australia	Springs	Allo, mtDNA	Deterministic	Restricted GF	–	–
	Austrochiltonia spp. A	Malacostraca	Low	Australia	Springs	Allo, mtDNA	Deterministic	Restricted GF	No GF	–
	Austrochiltonia spp. B	Malacostraca	Low	Australia	Springs	Allo, mtDNA	Deterministic	Restricted GF	No GF	–
	Austrochiltonia spp. C	Malacostraca	Low	Australia	Springs	Allo, mtDNA	Deterministic	Restricted GF	Restricted GF	–
	Austrochiltonia spp. F	Malacostraca	Low	Australia	Springs	Allo, mtDNA	Deterministic	Restricted GF	–	–
	Phreatochiltonia anophthalma	Malacostraca	Low	Australia	Springs	Allo, mtDNA	Deterministic	No GF	–	–
Phillipsen & Metcalf 2009	*Pseudacris cadaverina*	Amphibia	Low	USA	Rivers	mtDNA	Deterministic	Restricted GF	Restricted GF	No GF

Source	Species	Class	Disp	Study Location	Habitat Type	Markers Used	Analytical Method	Connectivity Model Concluded		
								Within Systems	Between Systems	Between Basins
Pritchard *et al.* 2009	*Oncorhynchus clarkii*	Osteichthyes	Mod	USA	Rivers	mtDNA, Msats	Probabilistic	SHM	No GF	No GF
Sei *et al.* 2009	*Gambusia nobilis*	Osteichthyes	Mod	USA	Springs	Allo	Deterministic	Restricted GF	–	–
	Gammarus pecos	Malacostraca	Low	USA	Springs	Allo	Deterministic	IBD	–	–
Seidel *et al.* 2009	*Gammarus* sp. "roswell"	Malacostraca	Low	USA	Springs	mtDNA	Deterministic	Restricted GF	–	–
	Gammarus sp. "toyah"	Malacostraca	Low	USA	Springs	mtDNA	Deterministic	Restricted GF	–	–
Wang 2009	*Bufo exsul*	Amphibia	Low	USA	Springs	Msats	Probabilistic	Restricted GF	–	–
Faulks *et al.* 2010	*Macquaria ambigua*	Osteichthyes	Mod	Australia	Rivers	Msats	Probabilistic	High GF	Restricted GF	No GF
Billman *et al.* 2010	*Rhinichthys osculus*	Osteichthyes	Mod	USA	Rivers	mtDNA	Deterministic	–	–	Low GF
Henriques *et al.* 2010	*Squalius torgalensis*	Osteichthyes	Mod	Portugal	Rivers	mtDNA, Msats	Probabilistic	High GF	–	–
Jungels *et al.* 2010	*Bufo cognata*	Amphibia	Mod	USA	Rivers	Msats	Deterministic	IBD	IBD	–
Korn *et al.* 2010	*Triops baeticus*	Branchiopoda	High	Portugal, Spain	Multiple	mtDNA	Deterministic	–	High GF	High GF
	Triops gadensis	Branchiopoda	High	Spain	Multiple	mtDNA	Deterministic	–	High GF	High GF
	Triops vicenticus	Branchiopoda	High	Portugal	Multiple	mtDNA	Deterministic	–	–	No GF
Martin 2010	*Cyprinodon nevadensis*	Osteichthyes	Low	USA	Springs	mtDNA, Msats	Probabilistic	High GF	Low GF	–
Mock *et al.* 2010	*Anodonta californiensis/ nuttalliana*	Bivalvia	Mod	USA	Rivers	Msats, mtDNA	Deterministic	–	No GF	No GF
Murphy *et al.* 2010	*Fonscochlea accepta*	Gastropoda	Mod	Australia	Springs	mtDNA	Probabilistic	High GF	High GF	–
	Ngarwa dirge	Ostracoda	High	Australia	Springs	mtDNA	Probabilistic	High GF	High GF	–
	Phreatomerus latipes	Malacostraca	Low	Australia	Springs	mtDNA	Probabilistic	High GF	SHM	–
	Wangiannachiltonia guzikae	Malacostraca	Low	Australia	Springs	mtDNA	Probabilistic	No GF	No GF	–
Sousa *et al.* 2010	*Iberochondrostoma almacai*	Osteichthyes	Low	Portugal	Rivers	Msats, mtDNA	Deterministic	Panmixia	Restricted GF	–
Stutz *et al.* 2010	*Hyalella azteca*	Malacostraca	Low	USA	Springs	mtDNA, nDNA	Deterministic	No GF	No GF	No GF
Woods *et al.* 2010	*Retropinna semoni*	Osteichthyes	Low	Australia	Rivers	Allo, Msats, mtDNA	Probabilistic	Restricted GF	Low GF	–
Chaves-Campos *et al.* 2011	*Palaemonetes suttkusi*	Malacostraca	Mod	Mexico	Springs	mtDNA	Probabilistic	Restricted GF	–	Low GF
Huey *et al.* 2011	*Macquaria ambigua*	Osteichthyes	Mod	Australia	Rivers	Msats	Deterministic	Restricted GF	–	–
	Tandanus tandanus	Osteichthyes	Mod	Australia	Rivers	Msats	Deterministic	Panmixia	–	–
	Macrobrachium australiense	Malacostraca	Mod	Australia	Rivers	Msats	Deterministic	Restricted GF	–	–
Morales *et al.* 2011	*Orestias ascotanensis*	Osteichthyes	Mod	Chile	Springs	mtDNA	Probabilistic	Restricted GF	No GF	–
Small *et al.* 2011	*Oncorhynchus tshawytscha*	Osteichthyes	Mod	USA	Rivers	Msats	Probabilistic	High GF	Low GF	–
Guzik *et al.* 2012	*Phreatomerus latipes*	Malacostraca	Low	Australia	Springs	Allo, mtDNA	Probabilistic	Restricted GF	No GF	–
Lopes-Cunha *et al.* 2012	*Iberochondrostoma lemmingii*	Osteichthyes	Low	Portugal, Spain	Rivers	Msats, mtDNA	Deterministic	–	Restricted GF	No GF
McGaugh 2012	*Apalone atra*	Reptilia	Low	Mexico	Multiple	Msats	Deterministic	–	No GF	–
Murphy *et al.* 2012	*Trochidrobia minuta*	Gastropoda	Low	Australia	Springs	mtDNA, nDNA	Probabilistic	No GF	No GF	–
	Trochidrobia punicea	Gastropoda	Low	Australia	Springs	mtDNA, nDNA	Probabilistic	No GF	No GF	–

Source	Species	Class	Disp	Study Location	Habitat Type	Markers Used	Analytical Method	Connectivity Model Concluded		
								Within Systems	Between Systems	Between Basins
	Trochidrobia smithi	Gastropoda	Low	Australia	Springs	mtDNA, nDNA	Probabilistic	No GF	No GF	–
Schwenter *et al.* 2012	*Limnadopsis birchii*	Branchiopoda	High	Australia	Rivers	mtDNA, nDNA	Probabilistic	IBD	Panmixia	IBD
	Limnadopsis paratatei	Branchiopoda	High	Australia	Rivers	mtDNA, nDNA	Probabilistic	Panmixia	Panmixia	Panmixia
	Limnadopsis parvispinus	Branchiopoda	High	Australia	Rivers	mtDNA, nDNA	Probabilistic	Multiple	Panmixia	IBD
	Limnadopsis tatei	Branchiopoda	High	Australia	Rivers	mtDNA, nDNA	Probabilistic	Panmixia	Panmixia	Panmixia
Bartakova *et al.* 2013	*Nothobranchius furzeri*	Osteichthyes	Low	Mozambique	Pools	mtDNA, Msats	Probabilistic	IBD	–	IBD
Coghill *et al.* 2013	*Lepomis megalotis*	Osteichthyes	Mod	Mexico	Rivers	mtDNA	Probabilistic	–	Low GF	No GF
Hopken *et al.* 2013	*Catostomus discobolus*	Osteichthyes	Mod	USA	Rivers	mtDNA, Msats	Probabilistic	SHM	SHM	No GF
Nguema *et al.* 2013	*Biomphalaria pfeifferi*	Gastropoda	Low	Oman	Rivers	Msats	Probabilistic	Restricted GF	No GF	No GF
Murphy *et al.* 2013	*Wangiannachiltonia guzikae*	Malacostraca	Low	Australia	Springs	mtDNA, nDNA	Probabilistic	DVM	DVM	–
Van Leeuwen *et al.* 2013	*Physa acuta*	Gastropoda	High	Spain	Springs	Msats	Probabilistic	–	IBD	–
Robertson *et al.* 2014	*Wangiannachiltonia guzikae*	Malacostraca	Low	Australia	Springs	Msats	Probabilistic	SHM	–	–
Schwentner *et al.* 2014	*Eocyzicus* species	Branchiopoda	High	Australia	Rivers	mtDNA, nDNA	Deterministic	–	High GF	High GF
Sousa-Santos *et al.* 2014a	*Anaecypris hispanica*	Osteichthyes	Low	Portugal	Rivers	mtDNA, nDNA	Probabilistic	Restricted GF	No GF	No GF
Sousa-Santos *et al.* 2014b	*Iberochondrostoma olisiponensis*	Osteichthyes	Low	Portugal	Rivers	nDNA, mtDNA	Deterministic	No GF	–	–
Velo-Anton *et al.* 2014	*Crocodylus suchus*	Reptilia	Mod	Mauritania	Rivers	mtDNA, Msats	Probabilistic	High GF	Restricted GF	–
Phillipsen *et al.* 2015	*Abedus herberti*	Insecta	Low	USA	Rivers	Msats	Deterministic	–	No GF	–

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Appendix 1.2 – Connectivity Levels among Taxonomic Groupings

The connectivity levels of the three most-studied taxonomic groups (fish, crustaceans, molluscs), which showed no significant differences in connectivity patterns.

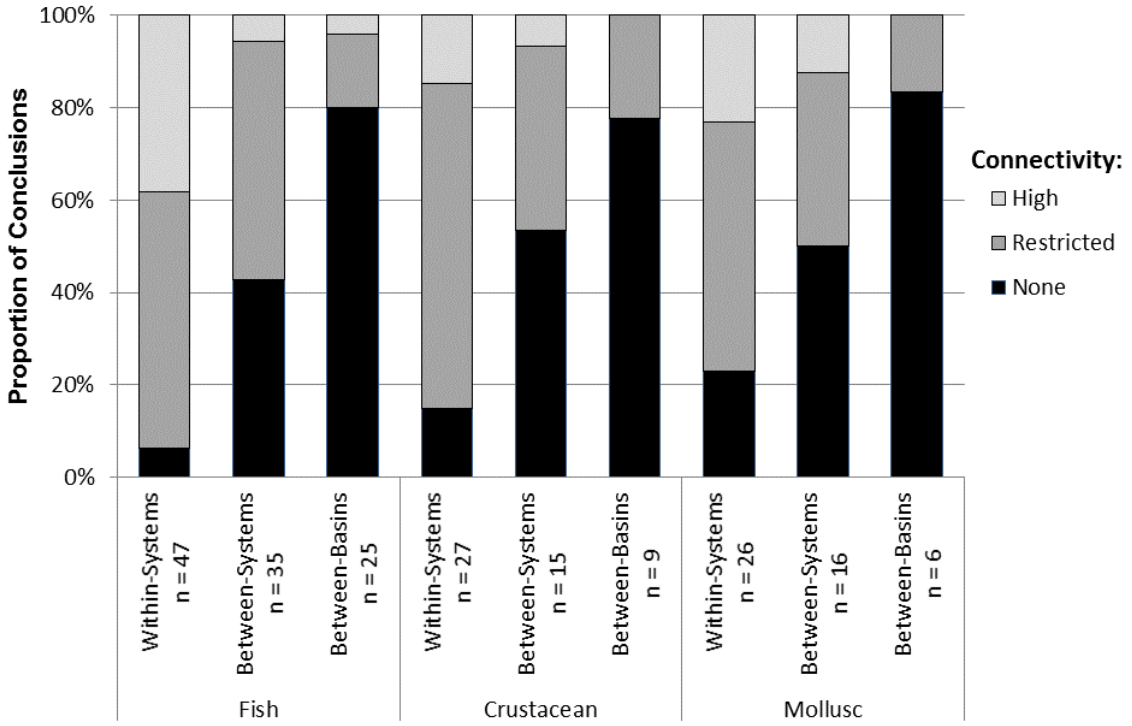

Figure 1. Proportion of studies of desert freshwater taxa that concluded three categories of connectivity, at three different scales, compared between the three most-studied taxonomic classes.

Appendix Two

Supporting Information for Chapter Five

Appendix 2.1 – Sampling Information

Details of locations and samples utilised in Chapter Five.

Table S1. Sampling sites in each of the six main river systems, and one major spring system, of the Lake Eyre Basin, including geographic coordinates and number of samples of each of five study taxa (G: Finke goby *Chlamydogobius japalpa* & desert goby *Chlamydogobius eremius*; H: Finke hardyhead *Craterocephalus centralis* & Lake Eyre hardyhead *Craterocephalus eyresii*; BG: barred grunter *Amniataba percoides*; SP: spangled perch *Leiopotherapon unicolor*; BH: bony herring *Nematalosa erebi*) included in this study. Site codes are mapped in Fig. 1 (lower case letters in site codes differentiate geographically close sites that are represented by a single dot on the map).

Site:	Latitude:	Longitude:	G	H	BG	SP	BH
Finke River							
F1 Ellery Creek Big Hole	23°46'39"S	133°04'24"E	-	-	10	5	-
F2 Giles Yard Spring	23°39'00"S	132°54'00"E	-	-	-	3	-
F3a Bowmans Gap	23°36'51"S	132°45'33"E	4	-	-	7	8
F3b Ormiston Gorge	23°37'44"S	132°43'39"E	-	-	3	3	8
F3c Pioneer Creek	23°40'53"S	132°43'21"E	9	-	-	-	-
F3d Two Mile Upper	23°40'09"S	132°40'11"E	9	11	8	10	-
F3e Glen Helen Gorge	23°41'13"S	132°40'25"E	10	14	7	10	-
F4 Boggy Hole	24°08'25"S	132°52'08"E	10	20	3	11	-
F5 Running Waters	24°18'29"S	132°54'10"E	3	10	9	1	5
F6a Three Mile Waterhole	24°30'50"S	133°13'18"E	-	5	10	-	10
F6b Maloney Creek	24°32'03"S	133°16'45"E	10	10	-	-	-
F6c Snake Hole	24°33'32"S	133°18'39"E	10	-	-	5	3
F7 Idracowra Station	25°00'09"S	133°47'32"E	1	-	2	-	-
F8 Horseshoe Bend Station	25°12'24"S	134°14'07"E	1	-	-	-	-
F9 Lilla Creek Station	25°27'05"S	134°13'30"E	-	-	-	2	1
Neales River							
N1 Stewart Waterhole	27°41'10"S	135°22'57"E	5	6	-	1	-
N2a Ockenden Spring	27°50'32"S	135°44'31"E	6	-	-	-	-
N2b Algebuckina Waterhole	27°54'00"S	135°48'52"E	5	10	5	1	7
N3 Peake Creek Rail Bridge	28°02'07"S	135°47'59"E	5	8	9	8	2
N4 Warrarawoona Waterhole	28°02'34"S	135°54'12"E	6	-	4	10	8
N5 Tardetakarinna Waterhole	28°00'55"S	136°08'16"E	6	-	-	-	-
N6 Levi Creek	28°19'03"S	136°16'15"E	10	-	-	-	-

	Latitude:	Longitude:	G	H	BG	SP	BH
South-West Creeks							
S1 Warriner Creek Crossing	29°08'16"S	136°34'06"E	7	7	-	-	-
S2 Margaret Creek Crossing	29°29'24"S	137°02'21"E	6	10	-	1	-
S3 Screechowl Creek Crossing	29°37'39"S	137°20'09"E	9	7	-	-	-
S4 Finniss Creek Crossing	29°36'36"S	137°27'29"E	10	10	-	-	-
Frome River							
Fr1 Leigh Creek Crossing	30°25'53"S	138°22'19"E	9	-	-	-	-
Fr2 Birdsville Track Crossing	29°38'47"S	138°04'16"E	-	3	-	1	4
Fr3 Muloorina Homestead	29°14'28"S	137°53'50"E	-	-	-	-	5
Cooper Creek							
C1 Lammermoor	21°20'35"S	144°38'51"E	-	-	-	5	9
C2 Agricultural College	23°21'04"S	144°19'45"E	-	-	-	5	4
C3 Stonehenge	24°21'02"S	143°15'22"E	-	-	-	5	5
C4 Avington Road	21°20'35"S	144°38'51"E	-	-	-	-	5
C5 Killman Waterhole	24°16'36"S	144°22'09"E	-	-	-	2	4
C6 Windorah Bridge	25°22'12"S	142°44'34"E	-	-	-	5	9
C7 One Mile Waterhole	25°50'37"S	143°03'07"E	-	-	-	5	-
C8 Durham Downs	27°03'08"S	141°54'13"E	-	-	-	-	9
C9 Yaningurie	28°57'54"S	140°07'05"E	-	-	-	2	-
C10 Tirra Warra Waterhole	27°26'00"S	140°08'58"E	-	-	-	-	9
C11 Lake Hope Camp	28°22'48"S	139°14'57"E	-	1	-	-	6
C12 Cuttapirra Waterhole	28°33'00"S	138°04'52"E	-	-	-	-	10
Georgina-Diamantina River System							
G1 Junction Waterhole	21°45'01"S	135°37'14"E	-	-	-	5	10
G2 Ooratippra Creek	22°21'43"S	135°39'17"E	-	-	-	5	10
G3 Lake Nash	20°58'04"S	137°55'53"E	-	-	-	-	10
G4 Tobermoray	22°16'26"S	137°58'36"E	-	-	-	-	10
G5 Thipinama Waterhole	22°47'06"S	137°56'57"E	-	-	-	2	1
G6 Police Barracks	22°43'11"S	140°01'54"E	-	-	5	10	10
G7 Bedourie	24°31'58"S	139°33'53"E	-	-	1	10	7
G8 Glengyle	24°49'40"S	139°37'17"E	-	-	-	-	1
G9 Mulligan River	23°55'54"S	138°38'24"E	-	5	-	-	-
G10 Oondoroo	22°10'37"S	143°09'49"E	-	-	-	-	1
G11 Davenport	24°09'24"S	141°06'08"E	-	-	-	-	10
G12 Brumbie Waterhole	25°39'17"S	139°50'12"E	-	-	-	-	8
G13 Pandie Pandie	26°07'27"S	139°23'11"E	-	-	-	1	-
G14 Bobbiemoonga Waterhole	26°35'59"S	139°33'17"E	-	-	-	-	3
G15 Ultoomurra Waterhole	27°09'14"S	138°43'35"E	8	5	1	-	-
G16a Cowarie Crossing	27°36'50"S	138°18'19"E	10	-	1	6	9
G16b Stoney Crossing	27°45'43"S	138°12'53"E	6	-	3	-	-
G17 Andaranna Waterhole	27°39'10"S	136°44'30"E	-	-	2	-	-
G18 Macumba Homestead	27°12'29"S	135°41'52"E	-	-	-	-	5
G19 Eringa Waterhole	26°17'15"S	134°43'45"E	-	-	-	4	3
Spring System							
DS Dalhousie Springs	26°25'21"S	135°30'11"E	-	-	-	5	-

Appendix 2.2 – Bioinformatics Details

Information on bioinformatics process, including custom R scripts used to filter SNPs extracted from sequence data and combine them into the genotype dataset. Details of final numbers of sequence and genotype loci used, and loci identified as being under selection, are provided for each taxa.

SNP Filter R Script

This R script, developed by Bertrand Gauffre and Ashley Murphy, filters an existing SNP table based on missing data and minor allele frequency. This was used with SNP tables outputted from adegenet, which identified variable sites from FASTAs. The SNP table (Transposed_ Final_SNP.csv) should be in the working directory, format the first column as loci ("loc") and the second as SNP position ("position"), and set the three thresholds at the start of the R script before running the filtering sections.

Genotyper R Script

To identify genotypes, this R script takes a SNP table outputted from the Adegenet package and converts it first into a list of genotypes per locus, and then gives each unique genotype at a locus a unique number (1-X). It then outputs a genotypic allele table, which looks like a SNP table, but with numbers ranging from 1 – X. Because it is unclear if individuals with missing data at some SNPs actually have unique genotypes, a conservative approach was taken to exclude all genotypes from the dataset that contained any missing data. This is because a genotypic allele of 0101NA is not unique if 01010 and 01011 already exist. So the unique identifiers solely refer to genotypic alleles with no missing data. The input (filtered) SNP table needs to be formatted to include an initial column of loci names (L1 – LX), labelled "locus" and then two columns for each individual, with SNPs coded as 0, 1 or NA.

SNP Filter R Script:

```
## SNP Filter - SCRIPT FOR SNP FILTERING
# The following script filters the SNP table in 3 successive
steps:
# 1) remove individuals with number of NA above a threshold
# 2) remove SNP with number of NA above a threshold
# 2) remove SNP with minor allelic frequency below a threshold

setwd("") # path of work directory

############### Load dataset
SNPloc<- as.data.frame(read.csv("HHAllSNPTable.csv", header=T,
sep=",", na.strings="NA"))

############### SET THRESHOLDS for individual and SNP filtering
in %
thresholdIND<-50 # Percentage of NA overall SNP above which an
                      individual is removed from dataset
thresholdLOC<-15 # the percentage of NA overall sequences under
                      which loci are kept
thresholdMINOR<-2 # the minor allelic frequency to remove (%)

############### INDIVIDUALS FILTERING BASED ON NA
thresholdIND2<-thresholdIND/100

############### APPLY FILTER (= remove columns)
nbloc<-nrow(SNPloc)
dataset_Filt_ind<-subset(SNPloc,
                         select= sapply(SNPloc, function(x)
sum(is.na(x))/nbloc<thresholdIND2))

############### SNP FILTERING BASED ON NA
nbind<-length(dataset_Filt_ind)-2     ## nbr column
dataset_Filt_ind$sumNA<-0
dataset_Filt_ind$pNA<-0
dataset_Filt_ind$sumALLELE<-0

############### SET THRESHOLD
thresholdLOC2<-thresholdLOC/100

############### APPLY FILTER (= remove rows)
for (i in 1:nrow(dataset_Filt_ind))
{
  dataset_Filt_ind$sumNA[i]<-
sum(is.na(dataset_Filt_ind[i,c(3:(nbind+2))]))
  dataset_Filt_ind$pNA[i]<-dataset_Filt_ind$sumNA[i]/nbind
}
dataset_Filt_indloc<-
dataset_Filt_ind[(dataset_Filt_ind[,"sumNA"]/nbind<thresholdLOC2),
]

############### Remove monomorphic SNP that could have appeared
after filtering on individuals
```

```
for (i in 1:nrow(dataset_Filt_indloc))
{
  dataset_Filt_indloc$sumALLELE[i]<-
      sum(dataset_Filt_indloc[i,c(3:(nbind+2))], na.rm=T)
  dataset_Filt_indloc$sumIndNoNA[i]<-nbind -
      dataset_Filt_indloc$sumNA[i]
  dataset_Filt_indloc$ratio[i]<-
      dataset_Filt_indloc$sumALLELE[i]/dataset_Filt_indloc$sumIndNo
NA[i]
}

dataset_Filt_indloc<-
      dataset_Filt_indloc[dataset_Filt_indloc[,"sumALLELE"]!=0 &
      dataset_Filt_indloc[,"ratio"]!=1,]

############## SNP FILTERING BASED ON MINOR ALLELIC FREQ
dataset_Filt_indloc$MinFreq<-
      dataset_Filt_indloc$sumALLELE/dataset_Filt_indloc$sumIndNoNA
thresholdMINOR<-thresholdMINOR
XXX<-thresholdMINOR/100
YYY<-1-(thresholdMINOR/100)
dataset_Filt_indlocminor<-
      dataset_Filt_indloc[dataset_Filt_indloc[,"MinFreq"]>XXX &
      dataset_Filt_indloc[,"MinFreq"]<YYY,]

############## RESULT OF FILTERING
write.csv(dataset_Filt_indlocminor, "FILT_loc_50-15-2.csv")
```

Genotyper R Script:

```
## Genotyper - SCRIPT FOR CREATING GENOTYPE DATA FROM SNPs
## Written by Bertrand Gauffre, 18/5/2016.
### Requires input matrix of SNPs (inds as columns and SNPs as
rows) ### The first column must be named "locus" and contain the
locus ID

setwd("")     # set the working directory
SNP<- read.csv("test.csv", header=T, sep=",")

## First, split SNP table by locus (i.e. create one table per
locus)
SNPbyLOC<-split(SNP, SNP$locus)

## A function to do the job for each locus
SNPtoHap<-function(x){
  # remove the column "loci"
  Locb<-x[,-1]
  # transposition of the datatable
  TrLocb <- as.data.frame(t(Locb))
  # A new column with SNP concatenated (ie haplotype)
  cols<-c(1:length(TrLocb))
  TrLocb$haplotype<- do.call(paste,c(TrLocb[cols], sep=""))
  # create a column with ind label
  TrLocb$label<-names(Locb)
  TrLocbCLEAN<-na.omit(TrLocb)
  # create list of all existing unique haplotypes
  haploLoc<-unique(TrLocbCLEAN$haplotype)
  truc<-data.frame(haploLoc)
  truc$haploID<-c(1:nrow(truc)) # attribute an ID (number) to each
     # different haplotype. merge dataset with SNP concatenated
     # (i.e. haplotype) with the list of unique haplotypes.
  locus<-merge(TrLocb, truc, by.x= "haplotype" , by.y= "haploLoc",
     all.x=  TRUE)
  Final<-locus[,c("label", "haploID")]
  Final2<-Final[order(Final$label),]
  return(Final2)
}

## Apply the function to the list of loci
good<-lapply(SNPbyLOC,SNPtoHap)
aa<-as.data.frame(good)

## Remove duplicated labels column
colum<-(1:length(aa))
test<-data.frame(colum)
test$pair<-colum%%2
vectorPair<-as.vector(test[(test$pair==0),1])

## Final output
GenotypeTable<-aa[,c(1,vectorPair)]
write.csv(GenotypeTable, "Genotypes.csv")
```

Table 1. Numbers and attributes of loci in the neutral sequence and genotype data sets, and the number of putatively non-neutral loci removed from each data set, for each of the five fish taxa. Lengths measured in number of base pairs (bp).

	Goby	Hardyhead	Grunter	Perch	Herring
Sequence Data Set					
No. of Loci	770	892	746	891	753
No. of Monomorphic Loci	64	138	168	243	98
Mean Sequence Length (bp)	578.3	586.4	674.2	578.9	566.6
Minimum Sequence Length (bp)	341	152	327	258	309
Maximum Sequence Length (bp)	768	744	1141	873	713
Genotype Data Set					
No. of Loci	706	754	578	648	655
Mean Genotypes per Locus	4.52	4.35	4.69	6.10	6.15
Minimum Genotypes per Locus	2	2	2	2	2
Maximum Genotypes per Locus	15	16	16	15	19
Non-neutral Loci					
No. Non-neutral Loci Removed	9	4	2	7	20

Table 2. The total number (and proportion; %) and identifiers of loci identified as being putatively under selection by BayeScan analyses for each of five fish taxa.

Taxa	Total	Loci
Goby	9 (1.2%)	L86 L98 L165 L292 L339 L453 L566 L620 L738
Hardyhead	4 (0.5%)	L532 L605 L700 L97
Barred Grunter	2 (0.3%)	L534 L554
Spangled Perch	7 (1.0%)	L101 L181 L190 L27 L377 L442 L840
Bony Herring	20 (2.8%)	L107 L118 L137 L237 L249 L28 L291 L408 L458 L563 L581 L590 L65 L727 L74 L806 L808 L822 L952 L987

Appendix 2.3 – Genetic Diversity Statistics

The following tables (Tables 1-5) present the waterhole scale levels of diversity within each sampled river of the Lake Eyre Basin for each of five fish taxa.

Table 1. Genetic diversity of goby in each sampled waterhole (*n*: number of individuals; PA: proportion of total alleles found in that population; AR: mean allelic richness; H_O: observed heterozygosity; H_E: expected heterozygosity; F_{IS}: global F_{IS} and 95% confidence interval).

Waterhole	*n*	PA	AR	H_O	H_E	F_{IS}	F_{IS} 95% CI	
Finke River								
F3a Bowmans Gap	4	29.55	0.96	0.014	0.014	-0.037	-0.486	0.681
F3c Pioneer Creek	9	29.93	1.01	0.013	0.013	-0.068	-0.294	0.132
F3d Two Mile Upper	9	30.35	1.04	0.024	0.022	-0.130	-0.305	-0.003
F3e Glen Helen Gorge	10	30.31	1.04	0.025	0.021	-0.235	-0.375	-0.140
F4 Boggy Hole	10	31.32	1.04	0.027	0.025	-0.051	-0.189	0.062
F5 Running Waters	3	29.57	1.02	0.017	0.014	-0.178	-0.461	0.451
F6b Maloney Creek	10	31.40	1.05	0.031	0.033	0.055	-0.094	0.172
F6c Snake Hole	10	31.16	1.06	0.036	0.032	-0.113	-0.219	-0.046
F7 Idracowra Station	2	30.04	1.03	0.037	0.027	-0.387	-0.732	0.103
Neales River								
N1 Stewart Waterhole	5	42.75	1.36	0.198	0.186	-0.061	-0.331	0.056
N2a Ockenden Spring	6	52.80	1.22	0.220	0.265	0.169	-0.227	0.559
N2b Algebuckina Waterhole	5	40.90	1.22	0.174	0.166	-0.055	-0.310	0.075
N3 Peake Creek Rail Bridge	5	40.11	1.10	0.163	0.166	0.020	-0.188	0.634
N4 Warrarawoona Waterhole	6	43.45	1.25	0.177	0.191	0.077	-0.074	0.167
N5 Tardetakarinna WH	6	43.63	1.35	0.197	0.187	-0.049	-0.234	0.041
N6 Levi Creek	10	44.91	1.33	0.184	0.183	-0.009	-0.093	0.044
South-West Creeks								
S1 Warriner Creek	7	56.37	1.58	0.327	0.330	0.008	-0.123	0.087
S2 Margaret Creek	6	50.31	1.25	0.277	0.291	0.050	-0.100	0.183
S3 Screechowl Creek	9	57.29	1.65	0.340	0.336	-0.013	-0.095	0.035
S4 Finniss Creek	10	57.90	1.66	0.357	0.347	-0.029	-0.103	0.016
Frome River								
Fr1 Leigh Creek Crossing	9	41.92	1.16	0.137	0.165	0.172	0.051	0.238
Georgina-Diamantina River								
G15 Ultoomurra Waterhole	8	35.36	1.09	0.086	0.090	0.042	-0.136	0.163
G16a Cowarie Crossing	10	46.56	1.34	0.157	0.202	0.223	0.072	0.294
G16b Stoney Crossing	6	36.30	1.16	0.104	0.102	-0.026	-0.207	0.062

Table 2. Genetic diversity of hardyhead in each sampled waterhole (*n*: number of individuals; PA: proportion of total alleles found in that population; AR: mean allelic richness; H_O: observed heterozygosity; H_E: expected heterozygosity; F_{IS}: global F_{IS} and 95% confidence interval).

Waterhole	*n*	PA	AR	H_O	H_E	F_{IS}	F_{IS} 95% CI	
Finke River								
F3d Two Mile Upper	11	47.42	1.35	0.177	0.205	0.136	0.044	0.194
F3e Glen Helen Gorge	14	49.23	1.44	0.192	0.213	0.097	0.041	0.133
F4 Boggy Hole	20	50.47	1.48	0.204	0.213	0.039	-0.004	0.075
F5 Running Waters	10	47.88	1.47	0.207	0.210	0.011	-0.067	0.058
F6a Three Mile Waterhole	5	45.50	1.44	0.219	0.199	-0.101	-0.373	0.033
F6b Maloney Creek	10	48.42	1.47	0.201	0.205	0.021	-0.054	0.073
Neales River								
N1 Stewart Waterhole	6	57.74	1.69	0.325	0.315	-0.030	-0.229	0.086
N2b Algebuckina Waterhole	10	64.29	1.82	0.338	0.335	-0.009	-0.077	0.037
N3 Peake Creek Rail Bridge	8	61.58	1.75	0.315	0.326	0.036	-0.081	0.103
South-West Creeks								
S1 Warriner Creek	7	64.47	1.85	0.346	0.345	0.000	-0.143	0.076
S2 Margaret Creek	10	68.80	1.89	0.361	0.365	0.012	-0.062	0.055
S3 Screechowl Creek	7	56.02	1.73	0.342	0.301	-0.132	-0.362	-0.030
S4 Finniss Creek	10	67.15	1.92	0.371	0.362	-0.027	-0.123	0.037
Frome River								
Fr2 Birdsville Crossing	3	50.94	1.65	0.327	0.283	-0.158	-0.502	0.183
Georgina-Diamantina River								
G9 Mulligan River	5	47.04	1.44	0.237	0.224	-0.061	-0.348	0.079
G15 Ultoomurra Waterhole	5	53.64	1.66	0.309	0.289	-0.066	-0.319	0.065

Table 3. Genetic diversity of barred grunter in each sampled waterhole (*n*: number of individuals; PA: proportion of total alleles found in that population; AR: mean allelic richness; H_O: observed heterozygosity; H_E: expected heterozygosity; F_{IS}: global F_{IS} and 95% confidence interval).

Waterhole	*n*	PA	AR	H_O	H_E	F_{IS}	F_{IS} 95% CI	
Finke River								
F1 Ellery Creek Big Hole	10	48.98	1.41	0.232	0.236	0.018	-0.072	0.077
F3b Ormiston Gorge	3	40.98	1.24	0.192	0.192	-0.002	-0.551	1.057
F3d Two Mile Upper	8	47.45	1.38	0.225	0.228	0.014	-0.097	0.076
F3e Glen Helen Gorge	7	47.24	1.38	0.227	0.224	-0.014	-0.164	0.066
F4 Boggy Hole	3	42.50	1.37	0.225	0.204	-0.100	-0.624	0.069
F5 Running Waters	9	48.50	1.42	0.231	0.232	0.001	-0.088	0.058
F6a Three Mile Waterhole	10	48.84	1.43	0.239	0.233	-0.027	-0.114	0.027
F7 Idracowra Station	2	26.73	1.03	0.137	0.311	0.661	0.584	0.821
Neales River								
N2b Algebuckina Waterhole	5	56.07	1.31	0.334	0.370	0.099	-0.065	0.268
N3 Peake Creek Rail Bridge	9	68.60	1.62	0.388	0.412	0.058	-0.091	0.164
N4 Warrarawoona Waterhole	4	56.07	1.46	0.405	0.375	-0.071	-0.305	0.177
Georgina-Diamantina								
G6 Police Barracks	5	66.25	1.80	0.439	0.428	-0.026	-0.199	0.100
G16b Stoney Crossing	3	45.63	1.19	0.320	0.351	0.145	-0.053	0.673
G17 Andaranna Waterhole	2	35.53	1.03	0.305	0.320	0.178	-0.036	0.573

Table 4. Genetic diversity of spangled perch in each sampled waterhole (*n*: number of individuals; PA: proportion of total alleles found in that population; AR: mean allelic richness; H_O: observed heterozygosity; H_E: expected heterozygosity; F_{IS}: global F_{IS} and 95% confidence interval).

Waterhole	*n*	PA	AR	H_O	H_E	F_{IS}	F_{IS} 95% CI	
Finke River								
F1 Ellery Creek Big Hole	5	38.18	1.40	0.271	0.260	-0.042	-0.288	0.082
F2 Giles Yard Spring	3	30.01	1.03	0.216	0.241	0.158	-0.020	0.631
F3a Bowmans Gap	7	40.45	1.20	0.261	0.292	0.110	-0.046	0.415
F3b Ormiston Gorge	3	35.27	1.32	0.269	0.242	-0.107	-0.472	0.730
F3d Two Mile Upper	10	42.66	1.50	0.280	0.286	0.021	-0.055	0.074
F3e Glen Helen Gorge	10	42.36	1.54	0.288	0.286	-0.009	-0.082	0.034
F4 Boggy Hole	12	42.32	1.50	0.272	0.280	0.029	-0.046	0.092
F6c Snake Hole	5	39.83	1.49	0.283	0.273	-0.035	-0.335	0.092
F9 Lilla Creek Station	2	28.66	1.08	0.235	0.248	0.129	-0.042	0.626
Neales River								
N2b Algebuckina Waterhole	2	33.61	1.27	0.336	0.244	-0.365	-0.950	0.572
N3 Peake Creek Rail Bridge	8	51.25	1.62	0.374	0.395	0.052	-0.077	0.138
N4 Warrarawoona Waterhole	10	53.55	1.75	0.407	0.411	0.011	-0.067	0.064
Cooper Creek								
C1 Lammermoor	5	49.55	1.75	0.420	0.387	-0.084	-0.353	0.035
C2 Agricultural College	5	50.34	1.75	0.416	0.395	-0.053	-0.342	0.073
C3 Stonehenge	5	49.10	1.75	0.420	0.388	-0.085	-0.367	0.040
C5 Killman Waterhole	2	38.74	1.60	0.430	0.330	-0.289	-0.519	-0.169
C6 Windorah Bridge	5	49.97	1.75	0.427	0.391	-0.092	-0.355	0.031
C7 One Mile Waterhole	5	49.66	1.79	0.451	0.394	-0.142	-0.375	-0.013
C9 Yaningurie	2	37.42	1.52	0.412	0.307	-0.327	-0.649	0.044
Georgina-Diamantina River								
G1 Junction Waterhole	5	51.09	1.78	0.433	0.407	-0.065	-0.319	0.068
G2 Ooratippra Creek	5	49.10	1.74	0.418	0.381	-0.099	-0.367	0.020
G5 Thipinama Waterhole	2	39.76	1.64	0.460	0.340	-0.345	-0.739	-0.153
G6 Police Barracks	10	60.96	1.89	0.454	0.458	0.010	-0.063	0.060
G7 Bedourie	10	61.04	1.93	0.470	0.463	-0.016	-0.094	0.028
G16a Cowarie Crossing	6	50.44	1.37	0.348	0.410	0.154	-0.008	0.260
G19 Eringa Waterhole	4	45.54	1.47	0.389	0.376	-0.026	-0.147	0.136
Spring System								
DS Dalhousie Springs	5	35.59	1.35	0.222	0.213	-0.046	-0.246	0.078

Table 5. Genetic diversity of bony herring in each sampled waterhole (*n*: number of individuals; PA: proportion of total alleles found in that population; AR: mean allelic richness; H_O: observed heterozygosity; H_E: expected heterozygosity; F_{IS}: global F_{IS} and 95% confidence interval).

Waterhole	*n*	PA	AR	H_O	H_E	F_{IS}	F_{IS} 95% CI	
Finke River								
F3a Bowmans Gap	8	38.36	1.42	0.245	0.240	-0.021	-0.141	0.044
F3b Ormiston Gorge	8	38.86	1.29	0.235	0.243	0.033	-0.083	0.106
F5 Running Waters	5	36.08	1.42	0.245	0.230	-0.065	-0.283	0.075
F6a Three Mile Waterhole	10	39.08	1.45	0.255	0.252	-0.012	-0.115	0.062
F6c Snake Hole	3	33.43	1.24	0.255	0.225	-0.126	-0.283	0.394
Neales River								
N2b Algebuckina Waterhole	7	52.08	1.49	0.369	0.365	-0.012	-0.179	0.082
N3 Peake Creek Rail Bridge	2	36.80	1.50	0.389	0.281	-0.383	-0.760	-0.039
N4 Warrarawoona Waterhole	8	57.35	1.71	0.402	0.406	0.009	-0.125	0.082
Frome River								
Fr2 Birdsville Crossing	4	44.33	1.57	0.348	0.328	-0.065	-0.311	0.097
Fr3 Muloorina HS	5	49.56	1.73	0.403	0.365	-0.105	-0.358	0.010
Cooper Creek								
C1 Lammermoor	9	39.00	1.43	0.240	0.225	-0.067	-0.151	-0.019
C2 Agricultural College	4	39.67	1.50	0.301	0.268	-0.130	-0.339	0.012
C3 Stonehenge	5	39.92	1.47	0.281	0.250	-0.125	-0.398	-0.008
C4 Avington Road	5	39.59	1.31	0.261	0.256	-0.019	-0.289	0.217
C5 Killman Waterhole	4	38.43	1.45	0.272	0.252	-0.086	-0.314	0.056
C6 Windorah Bridge	9	45.76	1.54	0.283	0.283	-0.001	-0.097	0.053
C8 Durham Downs	9	47.80	1.53	0.305	0.301	-0.013	-0.111	0.049
C10 Tirra Warra Waterhole	9	46.80	1.53	0.281	0.288	0.023	-0.076	0.089
C11 Lake Hope Camp	6	43.64	1.51	0.296	0.291	-0.019	-0.212	0.085
C12 Cuttapirra Waterhole	10	49.06	1.59	0.311	0.303	-0.030	-0.100	0.011
Georgina-Diamantina River								
G1 Junction Waterhole	10	53.53	1.70	0.383	0.375	-0.024	-0.123	0.035
G2 Ooratippra Creek	10	50.34	1.62	0.353	0.337	-0.048	-0.134	0.004
G3 Lake Nash	10	61.99	1.87	0.446	0.429	-0.041	-0.112	0.004
G4 Tobermoray	10	64.01	1.78	0.481	0.447	-0.076	-0.179	-0.009
G6 Police Barracks	10	61.91	1.78	0.435	0.427	-0.019	-0.098	0.038
G7 Bedourie	7	58.74	1.80	0.428	0.427	0.001	-0.147	0.075
G11 Davenport	10	58.34	1.69	0.400	0.401	0.003	-0.084	0.057
G12 Brumbie Waterhole	8	57.12	1.72	0.387	0.397	0.027	-0.095	0.103
G14 Bobbiemoonga WH	3	44.26	1.63	0.412	0.357	-0.152	-0.419	0.114
G16a Cowarie Crossing	9	61.54	1.84	0.439	0.423	-0.038	-0.118	0.112
G18 Macumba HS	5	47.50	1.67	0.383	0.335	-0.142	-0.402	-0.023
G19 Eringa Waterhole	3	34.45	1.13	0.306	0.304	0.066	-0.082	0.523

Appendix 2.4 – Population Genetic Structure Analyses

The following tables present the among-river pairwise F_{ST} and G''_{ST} (Tables 1-5), and the within-river pairwise F_{ST} (Tables 6–10) and G''_{ST} (Tables 11–15) values for each fish taxa. The final table presents the results of Isolation By Distance analyses.

The genetic structure analyses identified one goby, sampled in the Neales River, as showing evidence of originating in the Finke. Further analyses showed that this individual contained 53 alleles that were not shared with any other individuals (0.075 private alleles per locus), which is a far higher number than observed in any other gobies (data not shown) and even some rivers (Table 3). As such, it is likely this does not represent a migrant, or descendent of a migrant, from the Finke River, but may instead belong to a different goby species within the LEB. There are several distinctive goby species within the LEB that are endemic to local spring-systems, although none that connect hydrologically to the Neales (Larson 1995; Wager & Unmack 2000). One possible explanation for this unexpected appearance is anthropogenic translocation, with tourists a potential vectors in this landscape. The Dalhousie Springs, which contain an endemic and genetically-distinct goby species, are located approximately 160 km away and are a popular tourist stop in the LEB. Visitors to the Springs could conceivably have collected and transported a goby south, and camped along one of several waterholes of the Neales River that are located near the main route south through this area (the Oodnadatta Track).

Table 1. Among-river pairwise F_{ST} (below diagonal) and G''_{ST} (above diagonal) values and significance (* <0.05, ** <0.01) for goby in each sampled river system in the Lake Eyre Basin.

	Finke	Neales	South-West	Frome	Geo-Dia
Finke	-	0.910*	0.914*	0.935*	0.936*
Neales	0.684**	-	0.716*	0.183*	0.234*
South-West	0.625**	0.348**	-	0.752*	0.757*
Frome River	0.673**	0.055*	0.260**	-	0.186*
Geo-Dia	0.759**	0.098*	0.372**	0.082*	-

Table 2. Among-river pairwise F_{ST} (below diagonal) and G''_{ST} (above diagonal) values and significance (* <0.05, ** <0.01) for hardyhead in each sampled river system in the Lake Eyre Basin.

	Finke	Neales	South-West	Frome	Geo-Dia
Finke	-	0.637*	0.638*	0.651*	0.665*
Neales	0.267**	-	0.167*	0.033*	0.057*
South-West	0.290**	0.063*	-	0.129*	0.163*
Frome	0.089**	0.026	0.033	-	0.070*
Geo-Dia	0.194*	0.032	0.065	0.071	-

Table 3. Among-river pairwise F_{ST} (below diagonal) and G''_{ST} (above diagonal) values and significance (* <0.05, ** <0.01) for grunter in each sampled river system in the Lake Eyre Basin.

	Finke	Neales	Geo-Dia
Finke	-	0.588*	0.542*
Neales	0.194**	-	0.113*
Geo-Dia	0.157**	0.053	-

Table 4. Among-river pairwise F_{ST} (below diagonal) and G''_{ST} (above diagonal) values and significance (* <0.05, ** <0.01) for perch in each sampled river system in the Lake Eyre Basin.

	Finke	Neales	Cooper	Geo-Dia
Finke	-	0.585*	0.508*	0.514*
Neales	0.205**	-	0.443*	0.133*
Cooper	0.196**	0.148	-	0.350*
Geo-Dia	0.197**	0.047	0.109	-

Table 5. Among-river pairwise F_{ST} (below diagonal) and G''_{ST} (above diagonal) values and significance (* <0.05, ** <0.01) for herring in each sampled river system in the Lake Eyre Basin.

	Finke	Neales	Frome	Cooper	Geo-Dia
Finke	-	0.352*	0.367*	0.482*	0.331*
Neales	0.130**	-	0.018	0.126*	0.039*
Frome	0.126**	0.026	-	0.098*	0.037*
Cooper	0.188**	0.029	0.022	-	0.142*
Geo-Dia	0.093**	0.013	0.010	0.065*	-

Table 6. Within-river pairwise F_{ST} values (Nei 1973) and significance (*p*-values, italicised) for goby in each sampled waterhole in the Lake Eyre Basin. Site codes are mapped in Fig. 1, and further details on site locations are available in Appendix 2.1.

	F3a	**F3c**	**F3d**	**F3e**	**F4**	**F5**	**F6b**	**F6c**	**F7**	**F8**	**N1**	**N2a**	**N2b**	**N3**	**N4**	**N5**	**N6**	**S1**	**S2**	**S3**	**S4**	**Fr1**	**G15**	**G16a**	**G16b**
F3a	-	*0.045*	*0.160*	*0.140*	*0.131*	*0.036*	*0.055*	*0.043*	*1.000*	*0.017*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.005*	*0.004*
F3c	0.153	-	*0.146*	*0.181*	*0.069*	*0.089*	*0.025*	*0.016*	*0.996*	*0.052*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.005*	*0.003*
F3d	0.095	0.098	-	*0.910*	*0.384*	*0.421*	*0.085*	*0.076*	*1.000*	*0.347*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.004*	*0.001*
F3e	0.099	0.090	0.022	-	*0.252*	*0.381*	*0.052*	*0.044*	*0.999*	*0.264*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.002*	*0.001*
F4	0.097	0.143	0.059	0.076	-	*0.250*	*0.068*	*0.068*	*1.000*	*0.501*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.002*	*0.001*
F5	0.155	0.118	0.055	0.059	0.076	-	*0.176*	*0.147*	*1.000*	*0.085*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.002*	*0.001*	*0.006*	*0.003*
F6b	0.133	0.189	0.124	0.140	0.122	0.089	-	*0.654*	*1.000*	*1.000*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.002*	*0.002*
F6c	0.149	0.223	0.139	0.159	0.129	0.100	0.036	-	*1.000*	*0.999*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.005*	*0.005*
F7	0.067	0.020	0.068	0.061	0.052	0.211	0.060	0.086	-	*1.000*	*0.001*	*0.003*	*0.001*	*0.001*	*0.001*	*0.001*	*0.002*	*0.006*	*0.001*	*0.014*	*0.022*	*0.005*	*0.003*	*0.048*	*0.010*
F8	0.190	0.151	0.061	0.070	0.047	0.124	0.002	0.010	0.120	-	*0.001*	*0.003*	*0.001*	*0.001*	*0.001*	*0.001*	*0.003*	*0.003*	*0.003*	*0.007*	*0.018*	*0.002*	*0.002*	*0.042*	*0.018*
N1	0.700	0.767	0.748	0.753	0.750	0.669	0.737	0.732	0.442	0.464	-	*0.327*	*0.192*	*0.128*	*0.255*	*0.469*	*0.304*	*0.001*	*0.001*	*0.005*	*0.006*	*0.136*	*0.026*	*0.705*	*0.071*
N2a	0.530	0.592	0.571	0.577	0.578	0.487	0.567	0.557	0.296	0.316	0.065	-	*0.274*	*0.108*	*0.189*	*0.414*	*0.317*	*0.003*	*0.002*	*0.004*	*0.007*	*0.162*	*0.068*	*0.657*	*0.145*
N2b	0.728	0.776	0.756	0.760	0.756	0.700	0.743	0.738	0.498	0.524	0.081	0.075	-	*0.104*	*0.214*	*0.288*	*0.183*	*0.001*	*0.001*	*0.001*	*0.002*	*0.117*	*0.020*	*0.540*	*0.049*
N3	0.719	0.752	0.730	0.732	0.729	0.691	0.717	0.710	0.518	0.543	0.101	0.118	0.112	-	*0.218*	*0.212*	*0.249*	*0.002*	*0.001*	*0.001*	*0.005*	*0.155*	*0.035*	*0.589*	*0.073*
N4	0.675	0.741	0.724	0.729	0.726	0.643	0.716	0.710	0.427	0.448	0.072	0.095	0.081	0.082	-	*0.412*	*0.261*	*0.002*	*0.002*	*0.002*	*0.005*	*0.195*	*0.048*	*0.716*	*0.109*
N5	0.675	0.756	0.740	0.746	0.743	0.642	0.731	0.727	0.406	0.428	0.052	0.059	0.073	0.083	0.059	-	*0.338*	*0.001*	*0.002*	*0.001*	*0.002*	*0.174*	*0.027*	*0.745*	*0.097*
N6	0.605	0.721	0.710	0.720	0.716	0.570	0.707	0.705	0.323	0.339	0.071	0.070	0.091	0.081	0.078	0.068	-	*0.002*	*0.002*	*0.001*	*0.002*	*0.141*	*0.032*	*0.563*	*0.097*
S1	0.544	0.644	0.630	0.639	0.640	0.503	0.632	0.626	0.282	0.298	0.369	0.330	0.386	0.385	0.383	0.376	0.395	-	*0.242*	*0.372*	*0.476*	*0.003*	*0.005*	*0.020*	*0.026*
S2	0.594	0.650	0.632	0.637	0.639	0.559	0.631	0.622	0.374	0.393	0.390	0.355	0.411	0.425	0.401	0.387	0.379	0.084	-	*0.175*	*0.235*	*0.004*	*0.003*	*0.024*	*0.024*
S3	0.501	0.624	0.613	0.625	0.624	0.459	0.617	0.613	0.237	0.249	0.356	0.322	0.365	0.359	0.370	0.369	0.403	0.068	0.100	-	*0.813*	*0.004*	*0.004*	*0.029*	*0.030*
S4	0.471	0.602	0.593	0.605	0.605	0.430	0.598	0.595	0.213	0.224	0.336	0.305	0.344	0.336	0.353	0.352	0.391	0.060	0.093	0.034	-	*0.003*	*0.003*	*0.023*	*0.042*
Fr1	0.662	0.746	0.733	0.740	0.737	0.632	0.728	0.724	0.403	0.422	0.142	0.133	0.153	0.134	0.128	0.131	0.138	0.434	0.431	0.428	0.413	-	*0.086*	*0.500*	*0.240*
G15	0.796	0.852	0.838	0.842	0.836	0.776	0.825	0.824	0.565	0.592	0.292	0.231	0.312	0.265	0.266	0.282	0.265	0.493	0.496	0.480	0.458	0.192	-	*0.265*	*0.783*
G16a	0.578	0.701	0.690	0.701	0.697	0.541	0.688	0.687	0.294	0.310	0.068	0.075	0.089	0.083	0.067	0.059	0.085	0.376	0.357	0.386	0.375	0.090	0.131	-	*0.478*
G16b	0.802	0.851	0.834	0.838	0.832	0.782	0.819	0.817	0.579	0.608	0.262	0.207	0.286	0.252	0.241	0.252	0.233	0.459	0.472	0.441	0.417	0.170	0.044	0.106	-

Table 7. Within-river pairwise F_{ST} values (Nei 1973) and significance (*p*-values, italicised) for hardyhead in each sampled waterhole in the Lake Eyre Basin. Site codes are mapped in Fig. 1, and further details on site locations are available in Appendix 2.1.

Site	F3d	F3e	F4	F5	F6a	F6b	N1	N2b	N3	S1	S2	S3	S4	Fr2	C11	G9	G15
F3d	-	*0.401*	*0.504*	*0.296*	*0.126*	*0.293*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.998*	*0.001*	*0.004*
F3e	0.037	-	*0.665*	*0.380*	*0.300*	*0.493*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*1.000*	*0.001*	*0.007*
F4	0.033	0.028	-	*0.789*	*0.515*	*0.737*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.003*	*0.999*	*0.002*	*0.007*
F5	0.046	0.038	0.025	-	*0.234*	*0.543*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.999*	*0.001*	*0.011*
F6a	0.063	0.045	0.035	0.053	-	*0.224*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.999*	*0.001*	*0.005*
F6b	0.047	0.035	0.027	0.035	0.055	-	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.997*	*0.001*	*0.005*
N1	0.344	0.310	0.269	0.319	0.332	0.322	-	*0.432*	*0.308*	*0.043*	*0.052*	*0.019*	*0.074*	*0.097*	*1.000*	*0.076*	*0.436*
N2b	0.334	0.326	0.312	0.316	0.283	0.320	0.043	-	*0.583*	*0.063*	*0.073*	*0.023*	*0.098*	*0.299*	*1.000*	*0.150*	*0.714*
N3	0.342	0.322	0.293	0.320	0.308	0.324	0.048	0.035	-	*0.056*	*0.070*	*0.020*	*0.092*	*0.226*	*1.000*	*0.111*	*0.557*
S1	0.345	0.321	0.290	0.321	0.312	0.325	0.106	0.091	0.097	-	*0.597*	*0.084*	*0.395*	*0.036*	*1.000*	*0.018*	*0.167*
S2	0.327	0.319	0.304	0.308	0.274	0.312	0.095	0.087	0.089	0.036	-	*0.146*	*0.532*	*0.090*	*1.000*	*0.050*	*0.291*
S3	0.383	0.360	0.327	0.364	0.358	0.368	0.141	0.124	0.131	0.088	0.073	-	*0.269*	*0.016*	*1.000*	*0.007*	*0.094*
S4	0.329	0.323	0.309	0.310	0.273	0.314	0.091	0.084	0.085	0.048	0.042	0.061	-	*0.128*	*0.999*	*0.058*	*0.312*
Fr2	0.328	0.274	0.220	0.298	0.362	0.303	0.086	0.054	0.062	0.113	0.089	0.146	0.085	-	*1.000*	*0.020*	*0.299*
C11	-0.007	-0.012	-0.010	-0.028	-0.067	-0.025	-0.106	-0.059	-0.078	-0.062	-0.041	-0.067	-0.042	-0.208	-	*1.000*	*1.000*
G9	0.393	0.350	0.298	0.374	0.419	0.379	0.124	0.099	0.111	0.179	0.151	0.207	0.146	0.170	-0.170	-	*0.160*
G15	0.359	0.321	0.276	0.334	0.361	0.339	0.075	0.050	0.064	0.119	0.100	0.154	0.099	0.103	-0.137	0.151	-

Table 8. Within-river pairwise F_{ST} values (Nei 1973) and significance (*p*-values, italicised) for barred grunter in each sampled waterhole in the Lake Eyre Basin. Site codes are mapped in Fig. 1, and further details on site locations are available in Appendix 2.1.

Site	F1	F3b	F3d	F3e	F4	F5	F6a	F7	N2b	N3	N4	G6	G7	G15	G16a	G16b	G19
F1	-	*0.283*	*0.964*	*0.833*	*0.537*	*0.955*	*0.947*	*0.993*	*0.001*	*0.001*	*0.001*	*0.001*	*0.084*	*0.251*	*0.202*	*0.155*	*0.026*
F3b	0.067	-	*0.218*	*0.224*	*0.032*	*0.322*	*0.401*	*0.997*	*0.001*	*0.008*	*0.001*	*0.001*	*0.002*	*0.015*	*0.013*	*0.074*	*0.001*
F3d	0.032	0.073	-	*0.700*	*0.387*	*0.856*	*0.903*	*0.990*	*0.003*	*0.001*	*0.003*	*0.001*	*0.045*	*0.179*	*0.141*	*0.157*	*0.027*
F3e	0.039	0.073	0.045	-	*0.348*	*0.824*	*0.824*	*0.995*	*0.002*	*0.004*	*0.002*	*0.001*	*0.026*	*0.155*	*0.096*	*0.115*	*0.018*
F4	0.051	0.133	0.061	0.065	-	*0.517*	*0.576*	*0.997*	*0.001*	*0.015*	*0.001*	*0.004*	*0.004*	*0.036*	*0.044*	*0.094*	*0.001*
F5	0.032	0.067	0.038	0.039	0.055	-	*0.964*	*0.988*	*0.003*	*0.002*	*0.001*	*0.003*	*0.083*	*0.240*	*0.186*	*0.182*	*0.057*
F6a	0.034	0.061	0.036	0.040	0.054	0.029	-	*0.984*	*0.003*	*0.004*	*0.005*	*0.001*	*0.114*	*0.331*	*0.234*	*0.230*	*0.063*
F7	-0.049	-0.169	-0.066	-0.072	-0.161	-0.057	-0.050	-	*0.100*	*0.268*	*0.095*	*0.221*	*0.705*	*0.931*	*0.898*	*0.467*	*0.666*
N2b	0.253	0.309	0.273	0.276	0.293	0.260	0.251	0.147	-	*0.426*	*0.297*	*0.279*	*0.328*	*0.709*	*0.427*	*0.424*	*0.117*
N3	0.267	0.226	0.271	0.266	0.219	0.267	0.265	0.104	0.090	-	*0.646*	*0.488*	*0.726*	*0.889*	*0.709*	*0.587*	*0.076*
N4	0.247	0.318	0.270	0.276	0.296	0.256	0.245	0.154	0.118	0.071	-	*0.415*	*0.366*	*0.768*	*0.491*	*0.415*	*0.118*
G6	0.253	0.266	0.269	0.269	0.249	0.259	0.250	0.125	0.126	0.092	0.116	-	*0.731*	*0.894*	*0.700*	*0.577*	*0.089*
G7	0.167	0.361	0.198	0.215	0.318	0.178	0.164	0.059	0.129	0.069	0.129	0.077	-	*0.939*	*0.299*	*0.538*	*0.069*
G15	0.121	0.262	0.144	0.155	0.226	0.129	0.119	0.011	0.069	0.039	0.066	0.044	-0.046	-	*0.785*	*0.743*	*0.078*
G16a	0.164	0.364	0.198	0.215	0.325	0.179	0.164	0.002	0.137	0.073	0.130	0.082	0.187	0.023	-	*0.433*	*0.095*
G16b	0.191	0.292	0.210	0.223	0.269	0.198	0.186	0.105	0.142	0.096	0.152	0.105	0.133	0.014	0.120	-	*0.094*
G19	0.156	0.290	0.181	0.192	0.253	0.166	0.154	0.054	0.235	0.508	0.251	0.442	0.681	0.978	0.558	0.558	-

Table 9. Within-river pairwise F_{ST} values (Nei 1973) and significance (*p*-values, italicised) for spangled perch in each sampled waterhole in the Lake Eyre Basin. Site codes are mapped in Fig. 1, and further details on site locations are available in Appendix 2.1.

	F1	**F2**	**F3a**	**F3b**	**F3d**	**F3e**	**F4**	**F5**	**F6c**	**F9**	**N1**	**N2b**	**N3**	**N4**	**S2**	**Fr2**
F1	-	*0.160*	*0.201*	*0.062*	*0.466*	*0.484*	*0.455*	*0.384*	*0.196*	*0.734*	*0.986*	*0.001*	*0.007*	*0.012*	*0.986*	*0.993*
F2	0.095	-	*0.242*	*0.079*	*0.534*	*0.534*	*0.464*	*0.152*	*0.235*	*0.334*	*0.932*	*0.001*	*0.002*	*0.007*	*0.940*	*0.972*
F3a	0.085	0.080	-	*0.150*	*0.486*	*0.631*	*0.540*	*0.302*	*0.323*	*0.474*	*0.969*	*0.003*	*0.002*	*0.004*	*0.978*	*0.995*
F3b	0.123	0.114	0.091	-	*0.347*	*0.466*	*0.319*	*0.025*	*0.128*	*0.167*	*0.998*	*0.001*	*0.003*	*0.010*	*1.000*	*1.000*
F3d	0.057	0.055	0.056	0.065	-	*0.959*	*0.702*	*0.702*	*0.696*	*0.738*	*0.981*	*0.022*	*0.001*	*0.003*	*0.996*	*0.998*
F3e	0.058	0.056	0.049	0.058	0.028	-	*0.680*	*0.717*	*0.761*	*0.797*	*0.982*	*0.019*	*0.002*	*0.001*	*0.994*	*0.996*
F4	0.061	0.060	0.053	0.071	0.045	0.047	-	*0.671*	*0.606*	*0.755*	*0.973*	*0.012*	*0.003*	*0.002*	*0.981*	*0.993*
F5	0.067	0.100	0.071	0.147	0.045	0.045	0.048	-	*0.234*	*0.603*	*0.994*	*0.001*	*0.025*	*0.072*	*0.999*	*0.998*
F6c	0.084	0.083	0.071	0.097	0.045	0.043	0.051	0.084	-	*0.404*	*0.969*	*0.002*	*0.003*	*0.009*	*0.960*	*0.985*
F9	0.043	0.076	0.059	0.095	0.043	0.041	0.044	0.052	0.068	-	*0.993*	*0.001*	*0.016*	*0.047*	*0.990*	*0.996*
N1	0.059	0.001	0.017	0.047	0.003	0.001	0.001	0.393	0.020	0.234	-	*0.999*	*0.990*	*0.990*	*1.000*	*1.000*
N2b	0.359	0.304	0.253	0.374	0.174	0.170	0.180	0.519	0.268	0.361	0.065	-	*0.461*	*0.731*	*1.000*	*1.000*
N3	0.215	0.270	0.266	0.225	0.262	0.259	0.270	0.154	0.254	0.174	0.042	0.065	-	*0.888*	*0.999*	*1.000*
N4	0.184	0.248	0.246	0.195	0.262	0.262	0.271	0.122	0.235	0.144	0.032	0.050	0.040	-	*0.998*	*0.999*
S2	0.053	0.013	0.026	0.037	0.010	0.008	0.008	0.321	0.010	0.195	0.945	0.474	0.031	0.023	-	*1.000*
Fr2	0.167	0.034	0.007	0.103	0.013	0.019	0.018	0.540	0.053	0.261	1.054	0.628	0.041	0.033	1.209	-
C1	0.249	0.274	0.268	0.260	0.240	0.235	0.247	0.193	0.257	0.198	0.023	0.186	0.191	0.173	0.008	0.033
C2	0.241	0.263	0.257	0.248	0.231	0.225	0.236	0.183	0.249	0.188	0.021	0.182	0.189	0.174	0.011	0.032
C3	0.249	0.268	0.265	0.258	0.233	0.229	0.242	0.191	0.253	0.194	0.019	0.188	0.196	0.178	0.005	0.019
C5	0.322	0.285	0.263	0.325	0.188	0.184	0.198	0.332	0.258	0.284	0.115	0.324	0.166	0.135	0.082	0.145
C6	0.246	0.263	0.260	0.253	0.231	0.226	0.239	0.184	0.249	0.188	0.016	0.183	0.190	0.171	0.007	0.032
C7	0.240	0.259	0.256	0.250	0.227	0.221	0.235	0.184	0.247	0.189	0.019	0.187	0.193	0.176	0.006	0.028
C9	0.337	0.293	0.269	0.339	0.194	0.190	0.202	0.361	0.268	0.301	0.119	0.345	0.172	0.140	0.100	0.148
G1	0.257	0.288	0.279	0.263	0.251	0.248	0.260	0.193	0.266	0.209	0.034	0.138	0.129	0.114	0.019	0.048
G2	0.269	0.314	0.297	0.283	0.273	0.272	0.283	0.211	0.289	0.222	0.030	0.166	0.167	0.146	0.012	0.043
G5	0.353	0.319	0.290	0.348	0.218	0.214	0.224	0.362	0.288	0.317	0.155	0.254	0.107	0.084	0.113	0.165
G6	0.165	0.218	0.226	0.172	0.235	0.233	0.243	0.102	0.206	0.126	0.017	0.060	0.080	0.070	0.011	0.021
G7	0.165	0.218	0.225	0.171	0.235	0.235	0.242	0.101	0.206	0.125	0.017	0.059	0.080	0.071	0.011	0.021
G13	0.330	0.267	0.230	0.345	0.152	0.147	0.160	0.482	0.238	0.311	0.401	0.403	0.111	0.085	0.318	0.440
G16a	0.237	0.245	0.247	0.233	0.205	0.200	0.209	0.181	0.227	0.182	0.026	0.124	0.118	0.103	0.018	0.047
G17	0.274	0.279	0.266	0.272	0.211	0.206	0.216	0.242	0.253	0.229	0.993	0.029	0.133	0.251	0.987	0.994
DS	0.347	0.398	0.346	0.382	0.331	0.329	0.347	0.354	0.379	0.317	0.986	0.002	0.003	0.002	0.977	0.985

Table 9. Cont.

	C1	C2	C3	C5	C6	C7	C9	G1	G2	G5	G6	G7	G13	G16a	G19	DS
F1	*0.005*	*0.005*	*0.007*	*0.003*	*0.012*	*0.017*	*0.008*	*0.031*	*0.024*	*0.004*	*0.137*	*0.162*	*0.016*	*0.051*	*0.001*	*0.001*
F2	*0.006*	*0.006*	*0.012*	*0.006*	*0.011*	*0.015*	*0.020*	*0.023*	*0.016*	*0.023*	*0.071*	*0.085*	*0.050*	*0.050*	*0.003*	*0.001*
F3a	*0.003*	*0.003*	*0.006*	*0.003*	*0.006*	*0.009*	*0.015*	*0.018*	*0.015*	*0.025*	*0.053*	*0.056*	*0.059*	*0.047*	*0.002*	*0.001*
F3b	*0.002*	*0.002*	*0.004*	*0.003*	*0.011*	*0.016*	*0.009*	*0.045*	*0.048*	*0.031*	*0.170*	*0.184*	*0.051*	*0.097*	*0.002*	*0.001*
F3d	*0.002*	*0.004*	*0.005*	*0.031*	*0.012*	*0.029*	*0.066*	*0.028*	*0.038*	*0.066*	*0.066*	*0.074*	*0.205*	*0.093*	*0.007*	*0.001*
F3e	*0.007*	*0.005*	*0.010*	*0.025*	*0.016*	*0.023*	*0.063*	*0.035*	*0.030*	*0.066*	*0.063*	*0.069*	*0.241*	*0.100*	*0.012*	*0.001*
F4	*0.005*	*0.007*	*0.009*	*0.022*	*0.016*	*0.023*	*0.052*	*0.035*	*0.029*	*0.072*	*0.065*	*0.075*	*0.202*	*0.102*	*0.010*	*0.001*
F5	*0.022*	*0.021*	*0.024*	*0.002*	*0.037*	*0.050*	*0.003*	*0.073*	*0.067*	*0.009*	*0.450*	*0.489*	*0.001*	*0.125*	*0.003*	*0.001*
F6c	*0.005*	*0.007*	*0.007*	*0.008*	*0.011*	*0.016*	*0.015*	*0.028*	*0.026*	*0.027*	*0.084*	*0.085*	*0.077*	*0.076*	*0.002*	*0.001*
F9	*0.018*	*0.028*	*0.027*	*0.006*	*0.041*	*0.049*	*0.021*	*0.063*	*0.064*	*0.021*	*0.353*	*0.363*	*0.045*	*0.129*	*0.002*	*0.001*
N1	*0.991*	*0.988*	*0.983*	*0.995*	*0.977*	*0.969*	*0.984*	*0.977*	*0.970*	*0.991*	*0.975*	*0.982*	*0.998*	*0.967*	*0.074*	*0.050*
N2b	*0.032*	*0.044*	*0.037*	*0.002*	*0.056*	*0.078*	*0.010*	*0.228*	*0.180*	*0.075*	*0.845*	*0.869*	*0.035*	*0.336*	*0.154*	*0.312*
N3	*0.026*	*0.033*	*0.027*	*0.055*	*0.049*	*0.062*	*0.119*	*0.285*	*0.175*	*0.482*	*0.700*	*0.711*	*0.510*	*0.380*	*0.105*	*0.254*
N4	*0.043*	*0.045*	*0.064*	*0.101*	*0.085*	*0.091*	*0.218*	*0.398*	*0.248*	*0.683*	*0.793*	*0.796*	*0.733*	*0.519*	*0.087*	*0.233*
S2	*0.997*	*0.998*	*1.000*	*0.998*	*0.991*	*0.979*	*0.992*	*0.974*	*0.968*	*0.993*	*0.977*	*0.990*	*0.998*	*0.951*	*0.044*	*0.005*
Fr2	*1.000*	*0.999*	*0.995*	*0.999*	*0.994*	*0.986*	*0.998*	*0.984*	*0.983*	*0.996*	*0.982*	*0.990*	*1.000*	*0.983*	*0.082*	*0.064*
C1	-	*0.573*	*0.538*	*0.399*	*0.623*	*0.695*	*0.401*	*0.139*	*0.127*	*0.198*	*0.339*	*0.383*	*0.391*	*0.276*	*0.187*	*0.303*
C2	0.071	-	*0.666*	*0.516*	*0.752*	*0.747*	*0.480*	*0.146*	*0.142*	*0.189*	*0.366*	*0.388*	*0.421*	*0.309*	*0.183*	*0.300*
C3	0.077	0.067	-	*0.513*	*0.775*	*0.813*	*0.550*	*0.159*	*0.168*	*0.217*	*0.395*	*0.417*	*0.425*	*0.322*	*0.192*	*0.301*
C5	0.092	0.080	0.086	-	*0.546*	*0.644*	*0.179*	*0.184*	*0.186*	*0.140*	*0.573*	*0.633*	*0.238*	*0.332*	*0.218*	*0.319*
C6	0.074	0.063	0.063	0.084	-	*0.805*	*0.600*	*0.181*	*0.169*	*0.241*	*0.397*	*0.440*	*0.461*	*0.354*	*0.185*	*0.297*
C7	0.071	0.065	0.060	0.082	0.061	-	*0.546*	*0.161*	*0.179*	*0.264*	*0.420*	*0.474*	*0.458*	*0.356*	*0.191*	*0.293*
C9	0.107	0.102	0.092	0.172	0.090	0.093	-	*0.139*	*0.167*	*0.111*	*0.572*	*0.611*	*0.172*	*0.336*	*0.227*	*0.329*
G1	0.203	0.199	0.207	0.200	0.203	0.206	0.208	-	*0.622*	*0.432*	*0.802*	*0.817*	*0.507*	*0.431*	*0.152*	*0.287*
G2	0.223	0.221	0.230	0.223	0.226	0.228	0.228	0.092	-	*0.377*	*0.667*	*0.701*	*0.550*	*0.446*	*0.178*	*0.315*
G5	0.200	0.199	0.204	0.279	0.200	0.202	0.302	0.136	0.171	-	*0.889*	*0.911*	*0.330*	*0.536*	*0.178*	*0.313*
G6	0.144	0.143	0.147	0.111	0.143	0.145	0.119	0.075	0.108	0.054	-	*0.940*	*0.914*	*0.741*	*0.087*	*0.204*
G7	0.141	0.141	0.145	0.108	0.142	0.143	0.115	0.077	0.109	0.053	0.026	-	*0.940*	*0.777*	*0.087*	*0.201*
G13	0.143	0.138	0.144	0.274	0.137	0.143	0.295	0.146	0.165	0.275	0.070	0.068	-	*0.797*	*0.160*	*0.291*
G16a	0.160	0.155	0.159	0.167	0.157	0.156	0.175	0.144	0.166	0.144	0.092	0.089	0.087	-	*0.122*	*0.221*
G19	0.023	0.032	0.028	0.015	0.052	0.067	0.041	0.168	0.133	0.141	0.622	0.630	0.219	0.319	-	*0.273*
DS	0.003	0.003	0.004	0.002	0.006	0.018	0.009	0.023	0.033	0.038	0.105	0.115	0.061	0.103	0.003	-

Table 10. Within-river pairwise F_{ST} values (Nei 1973) and significance (*p*-values, italicised) for bony herring in each sampled waterhole in the Lake Eyre Basin. Site codes are mapped in Fig. 1, and further details on site locations are available in Appendix 2.1.

	F3a	**F3b**	**F5**	**F6a**	**F6c**	**F9**	**N2b**	**N3**	**N4**	**Fr2**	**Fr3**	**C1**	**C2**	**C3**	**C4**	**C5**	**C6**	**C8**
F3a	-	*0.350*	*0.073*	*0.200*	*0.060*	*1.000*	*0.001*	*0.001*	*0.002*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.002*
F3b	0.045	-	*0.073*	*0.175*	*0.027*	*1.000*	*0.002*	*0.001*	*0.001*	*0.001*	*0.002*	*0.001*	*0.002*	*0.001*	*0.001*	*0.003*	*0.002*	*0.002*
F5	0.069	0.067	-	*0.412*	*0.018*	*1.000*	*0.001*	*0.001*	*0.004*	*0.001*	*0.002*	*0.001*	*0.001*	*0.002*	*0.001*	*0.001*	*0.004*	*0.002*
F6a	0.054	0.056	0.044	-	*0.208*	*1.000*	*0.004*	*0.001*	*0.002*	*0.001*	*0.002*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*
F6c	0.077	0.086	0.092	0.055	-	*1.000*	*0.002*	*0.001*	*0.002*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.002*
F9	0.023	0.017	0.051	0.025	0.113	-	*0.335*	*0.027*	*0.764*	*0.109*	*0.393*	*0.033*	*0.028*	*0.033*	*0.011*	*0.024*	*0.151*	*0.247*
N2b	0.166	0.183	0.175	0.165	0.177	0.049	-	*0.048*	*0.266*	*0.072*	*0.110*	*0.021*	*0.050*	*0.049*	*0.011*	*0.053*	*0.077*	*0.067*
N3	0.198	0.222	0.250	0.171	0.296	0.090	0.084	-	*0.182*	*0.007*	*0.040*	*0.005*	*0.002*	*0.002*	*0.005*	*0.005*	*0.039*	*0.055*
N4	0.159	0.173	0.152	0.155	0.143	0.035	0.054	0.061	-	*0.160*	*0.491*	*0.017*	*0.101*	*0.082*	*0.026*	*0.098*	*0.136*	*0.122*
Fr2	0.207	0.220	0.222	0.184	0.241	0.066	0.079	0.137	0.063	-	*0.056*	*0.010*	*0.019*	*0.023*	*0.009*	*0.016*	*0.065*	*0.107*
Fr3	0.184	0.205	0.199	0.178	0.197	0.046	0.068	0.089	0.044	0.083	-	*0.011*	*0.051*	*0.035*	*0.009*	*0.047*	*0.061*	*0.072*
C1	0.293	0.284	0.300	0.303	0.266	0.092	0.101	0.129	0.105	0.125	0.121	-	*0.059*	*0.132*	*0.131*	*0.098*	*0.167*	*0.119*
C2	0.241	0.256	0.275	0.226	0.282	0.094	0.086	0.151	0.071	0.108	0.089	0.080	-	*0.112*	*0.057*	*0.096*	*0.399*	*0.397*
C3	0.256	0.262	0.284	0.249	0.287	0.094	0.085	0.140	0.078	0.110	0.100	0.069	0.071	-	*0.132*	*0.156*	*0.536*	*0.540*
C4	0.245	0.254	0.278	0.233	0.295	0.114	0.114	0.159	0.098	0.139	0.119	0.067	0.082	0.067	-	*0.068*	*0.329*	*0.211*
C5	0.247	0.260	0.289	0.237	0.300	0.105	0.081	0.152	0.073	0.113	0.093	0.072	0.073	0.064	0.083	-	*0.280*	*0.410*
C6	0.247	0.241	0.238	0.246	0.211	0.064	0.079	0.092	0.069	0.084	0.081	0.065	0.048	0.042	0.051	0.055	-	*0.804*
C8	0.238	0.237	0.230	0.236	0.200	0.057	0.082	0.090	0.070	0.076	0.081	0.072	0.049	0.044	0.061	0.048	0.035	-
C10	0.238	0.239	0.240	0.244	0.204	0.057	0.071	0.088	0.068	0.071	0.073	0.070	0.042	0.040	0.058	0.039	0.043	0.036
C11	0.254	0.261	0.257	0.236	0.245	0.074	0.099	0.130	0.082	0.092	0.103	0.098	0.076	0.072	0.094	0.080	0.052	0.043
C12	0.238	0.239	0.224	0.237	0.179	0.044	0.083	0.082	0.070	0.073	0.072	0.098	0.045	0.057	0.071	0.053	0.054	0.042
G1	0.184	0.192	0.176	0.195	0.150	0.042	0.087	0.085	0.076	0.102	0.084	0.171	0.117	0.130	0.135	0.118	0.132	0.135
G2	0.208	0.211	0.196	0.222	0.172	0.054	0.097	0.099	0.097	0.121	0.107	0.187	0.139	0.151	0.152	0.139	0.151	0.158
G3	0.156	0.169	0.132	0.153	0.120	0.029	0.070	0.064	0.044	0.067	0.053	0.145	0.089	0.106	0.116	0.096	0.102	0.103
G4	0.139	0.157	0.127	0.138	0.116	0.028	0.070	0.061	0.044	0.064	0.049	0.126	0.079	0.093	0.107	0.084	0.090	0.092
G5	0.149	0.166	0.184	0.112	0.261	0.017	0.087	0.223	0.063	0.122	0.101	0.132	0.172	0.166	0.190	0.190	0.097	0.095
G6	0.141	0.156	0.132	0.149	0.124	0.031	0.064	0.061	0.046	0.071	0.053	0.127	0.087	0.095	0.108	0.088	0.095	0.099
G7	0.164	0.187	0.162	0.159	0.146	0.034	0.085	0.082	0.057	0.085	0.066	0.160	0.110	0.128	0.146	0.115	0.123	0.114
G8	0.157	0.176	0.198	0.125	0.292	0.016	0.087	0.238	0.061	0.132	0.109	0.140	0.185	0.172	0.204	0.201	0.103	0.097
G10	0.145	0.165	0.202	0.123	0.273	0.014	0.081	0.203	0.056	0.130	0.091	0.117	0.154	0.141	0.162	0.163	0.086	0.085
G11	0.162	0.171	0.139	0.156	0.133	0.030	0.067	0.064	0.044	0.058	0.052	0.121	0.077	0.090	0.103	0.084	0.079	0.082
G12	0.165	0.179	0.157	0.164	0.150	0.036	0.069	0.073	0.045	0.072	0.054	0.123	0.087	0.094	0.110	0.091	0.082	0.088
G14	0.182	0.204	0.202	0.153	0.241	0.070	0.096	0.153	0.060	0.121	0.088	0.138	0.144	0.144	0.165	0.150	0.097	0.098
G16a	0.167	0.182	0.144	0.155	0.129	0.029	0.072	0.071	0.044	0.065	0.054	0.150	0.089	0.110	0.125	0.098	0.104	0.099
G17	0.143	0.161	0.183	0.138	0.200	0.041	0.093	0.016	0.099	0.016	0.053	0.034	0.027	0.038	0.018	0.043	0.074	0.081
G18	0.197	0.213	0.208	0.189	0.220	0.062	0.095	0.020	0.189	0.023	0.076	0.010	0.009	0.015	0.008	0.011	0.036	0.031

Table 10. Cont.

	C10	C11	C12	G1	G2	G3	G4	G5	G6	G7	G8	G10	G11	G12	G14	G16a	G17	G18
F3a	*0.002*	*0.001*	*0.001*	*0.008*	*0.004*	*0.018*	*0.022*	*0.018*	*0.024*	*0.021*	*0.023*	*0.042*	*0.037*	*0.043*	*0.037*	*0.045*	*0.006*	*0.002*
F3b	*0.002*	*0.001*	*0.002*	*0.006*	*0.004*	*0.007*	*0.018*	*0.016*	*0.018*	*0.016*	*0.011*	*0.026*	*0.031*	*0.026*	*0.030*	*0.030*	*0.002*	*0.001*
F5	*0.003*	*0.002*	*0.001*	*0.007*	*0.006*	*0.030*	*0.028*	*0.011*	*0.038*	*0.023*	*0.011*	*0.011*	*0.043*	*0.044*	*0.020*	*0.059*	*0.002*	*0.001*
F6a	*0.001*	*0.001*	*0.001*	*0.002*	*0.002*	*0.012*	*0.019*	*0.036*	*0.011*	*0.013*	*0.038*	*0.044*	*0.024*	*0.026*	*0.038*	*0.034*	*0.003*	*0.003*
F6c	*0.002*	*0.001*	*0.002*	*0.012*	*0.006*	*0.030*	*0.035*	*0.002*	*0.041*	*0.024*	*0.003*	*0.003*	*0.041*	*0.031*	*0.008*	*0.067*	*0.001*	*0.001*
F9	*0.276*	*0.115*	*0.599*	*0.716*	*0.432*	*0.968*	*0.974*	*0.997*	*0.972*	*0.954*	*0.996*	*0.979*	*0.963*	*0.938*	*0.331*	*0.911*	*0.525*	*0.132*
N2b	*0.137*	*0.051*	*0.107*	*0.083*	*0.057*	*0.195*	*0.203*	*0.102*	*0.277*	*0.138*	*0.152*	*0.185*	*0.335*	*0.328*	*0.181*	*0.304*	*0.072*	*0.070*
N3	*0.057*	*0.014*	*0.102*	*0.100*	*0.054*	*0.272*	*0.322*	*0.002*	*0.329*	*0.170*	*0.003*	*0.011*	*0.385*	*0.288*	*0.041*	*0.319*	*0.118*	*0.110*
N4	*0.168*	*0.088*	*0.177*	*0.145*	*0.060*	*0.679*	*0.697*	*0.311*	*0.695*	*0.446*	*0.387*	*0.481*	*0.813*	*0.813*	*0.514*	*0.817*	*0.071*	*0.058*
Fr2	*0.142*	*0.054*	*0.162*	*0.058*	*0.032*	*0.239*	*0.306*	*0.029*	*0.256*	*0.172*	*0.048*	*0.052*	*0.495*	*0.343*	*0.126*	*0.459*	*0.104*	*0.100*
Fr3	*0.120*	*0.042*	*0.162*	*0.113*	*0.051*	*0.468*	*0.568*	*0.073*	*0.514*	*0.317*	*0.086*	*0.170*	*0.610*	*0.630*	*0.252*	*0.631*	*0.082*	*0.076*
C1	*0.144*	*0.051*	*0.063*	*0.008*	*0.006*	*0.015*	*0.035*	*0.033*	*0.053*	*0.019*	*0.036*	*0.079*	*0.077*	*0.095*	*0.082*	*0.065*	*0.093*	*0.136*
C2	*0.617*	*0.118*	*0.576*	*0.028*	*0.015*	*0.114*	*0.161*	*0.011*	*0.139*	*0.068*	*0.019*	*0.038*	*0.281*	*0.229*	*0.067*	*0.236*	*0.098*	*0.120*
C3	*0.666*	*0.170*	*0.351*	*0.023*	*0.018*	*0.069*	*0.105*	*0.013*	*0.117*	*0.059*	*0.018*	*0.050*	*0.214*	*0.193*	*0.070*	*0.156*	*0.098*	*0.119*
C4	*0.266*	*0.057*	*0.162*	*0.021*	*0.018*	*0.034*	*0.069*	*0.006*	*0.074*	*0.025*	*0.008*	*0.022*	*0.153*	*0.133*	*0.038*	*0.110*	*0.118*	*0.139*
C5	*0.664*	*0.101*	*0.393*	*0.047*	*0.023*	*0.081*	*0.138*	*0.009*	*0.145*	*0.073*	*0.012*	*0.032*	*0.243*	*0.216*	*0.077*	*0.213*	*0.092*	*0.120*
C6	*0.571*	*0.393*	*0.379*	*0.027*	*0.019*	*0.080*	*0.104*	*0.102*	*0.125*	*0.063*	*0.125*	*0.213*	*0.268*	*0.278*	*0.225*	*0.194*	*0.077*	*0.099*
C8	*0.780*	*0.622*	*0.650*	*0.018*	*0.010*	*0.064*	*0.107*	*0.102*	*0.102*	*0.063*	*0.126*	*0.212*	*0.266*	*0.254*	*0.227*	*0.220*	*0.075*	*0.104*
C10	-	*0.425*	*0.815*	*0.022*	*0.012*	*0.067*	*0.119*	*0.097*	*0.117*	*0.088*	*0.113*	*0.212*	*0.227*	*0.241*	*0.226*	*0.241*	*0.069*	*0.101*
C11	0.051	-	*0.635*	*0.009*	*0.004*	*0.049*	*0.069*	*0.021*	*0.066*	*0.048*	*0.039*	*0.060*	*0.206*	*0.194*	*0.113*	*0.234*	*0.105*	*0.126*
C12	0.036	0.043	-	*0.015*	*0.006*	*0.067*	*0.124*	*0.113*	*0.088*	*0.106*	*0.168*	*0.282*	*0.239*	*0.237*	*0.280*	*0.322*	*0.076*	*0.110*
G1	0.135	0.148	0.141	-	*0.187*	*0.360*	*0.312*	*0.295*	*0.454*	*0.279*	*0.301*	*0.489*	*0.320*	*0.370*	*0.367*	*0.398*	*0.076*	*0.092*
G2	0.159	0.170	0.173	0.071	-	*0.147*	*0.148*	*0.211*	*0.280*	*0.118*	*0.270*	*0.365*	*0.171*	*0.249*	*0.237*	*0.255*	*0.083*	*0.102*
G3	0.107	0.107	0.104	0.059	0.082	-	*0.942*	*0.687*	*0.938*	*0.857*	*0.765*	*0.725*	*0.829*	*0.802*	*0.713*	*0.880*	*0.070*	*0.063*
G4	0.089	0.096	0.087	0.062	0.085	0.029	-	*0.711*	*0.883*	*0.863*	*0.725*	*0.740*	*0.752*	*0.760*	*0.750*	*0.891*	*0.067*	*0.060*
G5	0.100	0.135	0.091	0.063	0.073	0.043	0.043	-	*0.750*	*0.361*	*0.011*	*0.006*	*0.756*	*0.585*	*0.155*	*0.790*	*0.098*	*0.100*
G6	0.098	0.108	0.105	0.055	0.069	0.031	0.036	0.043	-	*0.785*	*0.767*	*0.801*	*0.775*	*0.819*	*0.741*	*0.845*	*0.066*	*0.055*
G7	0.109	0.125	0.102	0.072	0.100	0.038	0.038	0.069	0.044	-	*0.465*	*0.556*	*0.675*	*0.669*	*0.593*	*0.874*	*0.086*	*0.083*
G8	0.103	0.140	0.090	0.069	0.076	0.043	0.045	0.196	0.046	0.067	-	*0.005*	*0.776*	*0.682*	*0.218*	*0.828*	*0.122*	*0.114*
G10	0.086	0.128	0.078	0.057	0.069	0.045	0.045	0.263	0.045	0.062	0.268	-	*0.832*	*0.817*	*0.298*	*0.824*	*0.092*	*0.103*
G11	0.089	0.089	0.088	0.079	0.100	0.042	0.048	0.048	0.049	0.057	0.052	0.048	-	*0.939*	*0.875*	*0.879*	*0.064*	*0.060*
G12	0.090	0.099	0.093	0.076	0.093	0.045	0.049	0.061	0.047	0.062	0.065	0.054	0.035	-	*0.845*	*0.871*	*0.072*	*0.061*
G14	0.100	0.127	0.093	0.081	0.102	0.054	0.053	0.135	0.057	0.077	0.147	0.137	0.051	0.061	-	*0.845*	*0.127*	*0.102*
G16a	0.097	0.100	0.087	0.078	0.106	0.036	0.032	0.049	0.045	0.042	0.051	0.053	0.044	0.050	0.054	-	*0.074*	*0.070*
G17	0.132	0.034	0.149	0.151	0.113	0.211	0.251	0.075	0.271	0.147	0.054	0.146	0.395	0.318	0.094	0.304	-	*0.063*
G18	0.037	0.012	0.026	0.067	0.056	0.282	0.342	0.073	0.443	0.155	0.053	0.099	0.442	0.443	0.150	0.299	0.077	-

Table 11. Within-river pairwise G''_{ST} values (Meirmans & Hedrick 2011) and significance (based on 95% credible intervals, * denotes intervals that excluded zero; ~ denotes intervals which could not be calculated) for goby in each sampled waterhole in the Lake Eyre Basin. Only waterholes with three or more individuals sampled included. Site codes are mapped in Fig. 1, and further details on site locations are available in Appendix 2.1.

	F3a	F3c	F3d	F3e	F4	F5	F6b	F6c	N1	N2a	N2b	N3	N4	N5	N6	S1	S2	S3	S4	Fr1	G15	G16a	G16b
F3a	-	*	*	*	ns	ns	*	*	*	*	*	~	~	*	*	*	~	*	*	*	~	*	*
F3c	0.244	-	ns	ns	*	ns	*	*	*	*	*	~	~	*	*	*	~	*	*	*	~	*	*
F3d	0.097	0.116	-	ns	ns	ns	*	*	*	*	*	~	~	*	*	*	~	*	*	*	~	*	*
F3e	0.116	0.105	0.012	-	*	ns	*	*	*	*	*	~	~	*	*	*	~	*	*	*	~	*	*
F4	0.094	0.205	0.063	0.096	-	ns	*	*	*	*	*	~	~	*	*	*	~	*	*	*	~	*	*
F5	0.061	0.147	0.032	0.057	0.116	-	*	*	*	*	*	~	~	*	*	*	~	*	*	*	~	*	*
F6b	0.196	0.288	0.191	0.221	0.178	0.161	-	ns	*	*	*	~	~	*	*	*	~	*	*	*	~	*	*
F6c	0.230	0.326	0.209	0.242	0.186	0.184	0.020	-	*	*	*	~	~	*	*	*	~	*	*	*	~	*	*
N1	0.923	0.927	0.924	0.924	0.923	0.921	0.919	0.919	-	ns	ns	~	~	ns	*	*	~	*	*	*	~	ns	*
N2a	0.842	0.852	0.847	0.849	0.846	0.840	0.840	0.840	0.081	-	ns	~	~	ns	*	*	~	*	*	*	~	*	*
N2b	0.932	0.936	0.933	0.934	0.932	0.931	0.929	0.929	0.040	0.085	-	~	~	ns	*	*	~	*	*	*	~	ns	*
N3	0.930	0.934	0.931	0.931	0.930	0.929	0.926	0.926	0.063	0.117	0.083	-	~	~	~	~	~	~	~	~	~	~	~
N4	0.924	0.929	0.925	0.926	0.925	0.923	0.921	0.921	0.051	0.122	0.060	0.028	-	~	~	~	~	~	~	~	~	~	~
N5	0.923	0.927	0.924	0.925	0.923	0.922	0.920	0.920	0.009	0.082	0.035	0.031	0.025	-	*	*	~	*	*	*	~	ns	*
N6	0.927	0.931	0.928	0.929	0.927	0.926	0.924	0.924	0.092	0.147	0.135	0.092	0.110	0.087	-	*	~	*	*	*	~	*	*
S1	0.915	0.921	0.919	0.919	0.918	0.914	0.915	0.915	0.724	0.683	0.736	0.734	0.728	0.725	0.741	-	~	*	*	*	~	*	*
S2	0.925	0.930	0.928	0.929	0.928	0.924	0.924	0.925	0.744	0.703	0.754	0.757	0.748	0.747	0.762	0.060	-	~	~	~	~	~	~
S3	0.920	0.924	0.923	0.923	0.922	0.919	0.919	0.920	0.737	0.700	0.747	0.747	0.741	0.739	0.753	0.096	0.153	-	ns	*	~	*	*
S4	0.917	0.922	0.920	0.921	0.920	0.916	0.917	0.917	0.731	0.694	0.742	0.739	0.736	0.733	0.747	0.081	0.138	0.018	-	*	~	*	*
Fr1	0.937	0.940	0.937	0.938	0.936	0.936	0.933	0.933	0.210	0.227	0.247	0.204	0.205	0.195	0.238	0.761	0.780	0.772	0.766	-	~	*	*
G15	0.964	0.967	0.964	0.964	0.962	0.964	0.960	0.960	0.439	0.443	0.493	0.463	0.446	0.437	0.459	0.807	0.825	0.816	0.808	0.334	-	~	~
G16a	0.921	0.926	0.922	0.923	0.921	0.920	0.918	0.918	0.090	0.153	0.134	0.088	0.083	0.069	0.138	0.731	0.750	0.743	0.736	0.120	0.199	-	ns
G16b	0.960	0.963	0.960	0.960	0.959	0.960	0.956	0.956	0.408	0.419	0.459	0.431	0.417	0.405	0.433	0.799	0.816	0.809	0.801	0.311	0.014	0.176	-

Table 12. Within-river pairwise G''_{ST} values (Meirmans & Hedrick 2011) and significance (based on 95% credible intervals, * denotes intervals that excluded zero) for hardyhead in each sampled waterhole in the Lake Eyre Basin. Only waterholes with three or more individuals sampled included. Site codes are mapped in Fig. 1, and further details on site locations are available in Appendix 2.1.

	F3d	**F3e**	**F4**	**F5**	**F6a**	**F6b**	**N1**	**N2b**	**N3**	**S1**	**S2**	**S3**	**S4**	**Fr2**	**G9**	**G15**
F3d	-	ns	ns	ns	ns	ns	*	*	*	*	*	*	*	*	*	*
F3e	0.023	-	ns	ns	ns	ns	*	*	*	*	*	*	*	*	*	*
F4	0.037	0.028	-	ns	ns	ns	*	*	*	*	*	*	*	*	*	*
F5	0.036	0.036	0.019	-	ns	ns	*	*	*	*	*	*	*	*	*	*
F6a	0.061	0.045	0.051	0.047	-	ns	*	*	*	*	*	*	*	*	*	*
F6b	0.037	0.029	0.027	0.021	0.051	-	*	*	*	*	*	*	*	*	*	*
N1	0.651	0.650	0.654	0.646	0.642	0.651	-	ns	ns	*	*	*	*	ns	*	ns
N2b	0.642	0.641	0.648	0.636	0.632	0.642	0.016	-	ns	*	*	*	*	ns	*	ns
N3	0.647	0.647	0.652	0.642	0.637	0.647	0.014	0.015	-	*	*	*	*	ns	*	ns
S1	0.647	0.648	0.653	0.643	0.637	0.647	0.183	0.182	0.178	-	ns	*	ns	*	*	*
S2	0.641	0.645	0.648	0.639	0.635	0.644	0.186	0.183	0.176	0.013	-	*	ns	*	*	*
S3	0.696	0.698	0.701	0.693	0.691	0.698	0.274	0.272	0.268	0.146	0.132	-	*	*	*	*
S4	0.640	0.643	0.647	0.637	0.633	0.642	0.175	0.173	0.162	0.051	0.047	0.093	-	*	*	*
Fr2	0.653	0.652	0.659	0.649	0.647	0.655	0.050	0.040	0.026	0.193	0.189	0.284	0.173	-	*	*
G9	0.726	0.723	0.729	0.721	0.719	0.726	0.177	0.178	0.182	0.352	0.344	0.407	0.324	0.227	-	*
G15	0.664	0.664	0.670	0.660	0.659	0.666	0.062	0.047	0.066	0.221	0.214	0.304	0.208	0.088	0.220	-

Table 13. Within-river pairwise G''_{ST} values (Meirmans & Hedrick 2011) and significance (based on 95% credible intervals, * denotes intervals that excluded zero) for barred grunter in each sampled waterhole in the Lake Eyre Basin. Only waterholes with three or more individuals sampled included. Site codes are mapped in Fig. 1, and further details on site locations are available in Appendix 2.1.

	F1	**F3d**	**F3e**	**F4**	**F5**	**F6c**	**N3**	**G6**
F1	-	ns	ns	ns	ns	ns	*	*
F3d	-0.002	-	ns	ns	ns	ns	*	*
F3e	0.014	0.014	-	ns	ns	ns	*	*
F4	0.031	0.031	0.022	-	ns	ns	*	*
F5	0.008	0.011	0.014	0.028	-	ns	*	*
F6c	0.017	0.013	0.020	0.043	0.003	-	*	*
N3	0.613	0.614	0.611	0.600	0.612	0.613	-	*
G6	0.563	0.564	0.564	0.548	0.565	0.561	0.169	-

Table 14. Within-river pairwise G''_{ST} values (Meirmans & Hedrick 2011) and significance (based on 95% credible intervals, * denotes intervals that excluded zero; ~ denotes intervals which could not be calculated) for spangled perch in each sampled waterhole in the Lake Eyre Basin. Only waterholes with three or more individuals sampled included. Site codes are mapped in Fig. 1, and further details on site locations are available in Appendix 2.1.

	F1	F2	F3a	F3b	F3d	F3e	F4	F6c	F9	N2b	N3	N4	C1	C2	C3	C5	C6	C7	C9	G1	G2	G5	G6	G7	G16a	G17	DS
F1	-	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~
F2	0.157	-	~	~	ns	ns	ns	ns	~	~	*	*	*	~	*	~	~	*	~	*	*	~	*	*	~	~	~
F3a	0.148	0.072	-	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~
F3b	0.190	0.095	0.061	-	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~
F3d	0.132	0.047	0.060	0.059	-	ns	ns	ns	~	~	*	*	*	~	*	~	~	*	~	*	*	~	*	*	~	~	~
F3e	0.152	0.051	0.055	0.039	0.001	-	ns	ns	~	~	*	*	*	~	*	~	~	*	~	*	*	~	*	*	~	~	~
F4	0.143	0.055	0.062	0.077	0.040	0.045	-	ns	~	~	*	*	*	~	*	~	~	*	~	*	*	~	*	*	~	~	~
F6c	0.128	0.060	0.062	0.056	0.025	0.022	0.041	-	~	~	*	*	*	~	*	~	~	*	~	*	*	~	*	*	~	~	~
F9	0.225	0.125	0.099	0.111	0.116	0.112	0.123	0.117	-	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~
N2b	0.675	0.669	0.628	0.628	0.649	0.661	0.662	0.642	0.664	-	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~
N3	0.621	0.623	0.585	0.579	0.605	0.612	0.618	0.596	0.606	0.088	-	ns	*	~	*	~	~	*	~	*	*	~	*	*	~	~	~
N4	0.623	0.623	0.592	0.582	0.607	0.614	0.619	0.597	0.604	0.075	0.019	-	*	~	*	~	~	*	~	*	*	~	*	*	~	~	~
C1	0.578	0.559	0.532	0.541	0.548	0.552	0.560	0.544	0.521	0.493	0.462	0.465	-	~	ns	~	~	ns	~	*	*	~	*	*	~	~	~
C2	0.563	0.540	0.509	0.514	0.531	0.534	0.540	0.531	0.505	0.498	0.459	0.466	0.060	-	~	~	~	~	~	~	~	~	~	~	~	~	~
C3	0.567	0.549	0.521	0.532	0.537	0.543	0.552	0.536	0.515	0.502	0.477	0.479	0.069	0.044	-	~	~	ns	~	*	*	~	*	*	~	~	~
C5	0.590	0.562	0.537	0.542	0.547	0.553	0.565	0.547	0.540	0.515	0.470	0.471	0.062	0.019	0.035	-	~	~	~	~	~	~	~	~	~	~	~
C6	0.568	0.540	0.514	0.524	0.532	0.535	0.547	0.532	0.502	0.501	0.462	0.464	0.068	0.038	0.024	0.040	-	~	~	~	~	~	~	~	~	~	~
C7	0.555	0.530	0.507	0.515	0.523	0.524	0.536	0.522	0.498	0.506	0.470	0.475	0.050	0.036	0.010	0.017	0.016	-	~	*	*	~	*	*	~	~	~
C9	0.602	0.576	0.542	0.548	0.564	0.566	0.578	0.558	0.555	0.517	0.485	0.492	0.091	0.072	0.020	0.080	0.022	0.019	-	~	~	~	~	~	~	~	~
G1	0.604	0.598	0.567	0.570	0.580	0.591	0.596	0.576	0.572	0.328	0.280	0.282	0.472	0.464	0.480	0.481	0.474	0.481	0.491	-	*	~	*	*	~	~	~
G2	0.630	0.638	0.598	0.601	0.615	0.625	0.628	0.607	0.605	0.431	0.387	0.378	0.508	0.508	0.526	0.534	0.523	0.523	0.538	0.127	-	~	*	*	~	~	~
G5	0.640	0.633	0.594	0.592	0.619	0.628	0.627	0.611	0.611	0.255	0.220	0.211	0.455	0.457	0.460	0.455	0.460	0.456	0.491	0.216	0.345	-	~	~	~	~	~
G6	0.577	0.582	0.557	0.546	0.570	0.575	0.580	0.560	0.549	0.200	0.161	0.151	0.405	0.404	0.417	0.411	0.410	0.413	0.441	0.161	0.283	0.052	-	ns	~	~	~
G7	0.577	0.585	0.552	0.547	0.568	0.578	0.577	0.561	0.551	0.192	0.160	0.152	0.398	0.399	0.414	0.399	0.409	0.407	0.426	0.164	0.288	0.041	0.004	-	~	~	~
G16a	0.568	0.562	0.520	0.527	0.545	0.556	0.561	0.540	0.514	0.281	0.245	0.240	0.347	0.323	0.336	0.340	0.329	0.328	0.357	0.286	0.367	0.238	0.193	0.180	-	~	~
G17	0.589	0.586	0.546	0.529	0.564	0.572	0.577	0.560	0.543	0.224	0.183	0.180	0.393	0.388	0.409	0.404	0.396	0.403	0.415	0.275	0.363	0.241	0.180	0.179	0.149	-	~
DS	0.736	0.709	0.681	0.695	0.687	0.689	0.702	0.685	0.718	0.643	0.581	0.578	0.605	0.604	0.602	0.621	0.596	0.591	0.632	0.583	0.619	0.593	0.537	0.527	0.523	0.571	-

Table 15. Within-river pairwise G''_{ST} values (Meirmans & Hedrick 2011) and significance (based on 95% credible intervals, * denotes intervals that excluded zero) for bony herring in each sampled waterhole in the Lake Eyre Basin. Only waterholes with three or more individuals sampled included. Site codes are mapped in Fig. 1, and further details on site locations are available in Appendix 2.1.

	F3a	F3b	F5	F6a	N2b	N4	Fr2	F23	C1	C2	C3	C4	C5	C6	C8	C10	C11	C12	G1	G2	G3	G4	G6	G7	G11	G12	G16a	G18
F3a	-	ns	ns	ns	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
F3b	0.010	-	ns	ns	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
F5	0.072	0.051	-	ns	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
F6a	0.065	0.058	0.025	-	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
N2b	0.367	0.364	0.385	0.392	-	ns	ns	ns	*	*	*	*	*	*	*	*	*	*	*	*	*	*	ns	*	*	ns	*	ns
N4	0.351	0.346	0.351	0.355	0.018	-	ns	ns	*	*	*	*	*	*	*	*	*	*	*	*	ns	ns	ns	ns	ns	ns	ns	ns
Fr2	0.422	0.418	0.415	0.422	0.068	0.052	-	ns	*	ns	*	*	*	*	*	ns	ns	*	*	*	*	*	*	*	ns	ns	ns	ns
Fr3	0.364	0.370	0.383	0.384	0.043	0.006	0.058	-	*	ns	*	*	ns	*	*	*	*	*	*	*	ns	ns	ns	ns	ns	ns	ns	ns
C1	0.561	0.562	0.583	0.585	0.241	0.229	0.245	0.223	-	*	*	*	ns	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
C2	0.482	0.487	0.498	0.498	0.128	0.106	0.111	0.087	0.112	-	ns	ns	ns	ns	ns	ns	ns	ns	*	*	*	*	*	*	*	*	*	*
C3	0.501	0.500	0.522	0.523	0.150	0.136	0.139	0.128	0.085	0.023	-	ns	ns	ns	ns	ns	ns	ns	*	*	*	*	*	*	*	*	*	*
C4	0.498	0.498	0.513	0.518	0.167	0.125	0.156	0.116	0.089	0.012	0.008	-	ns	ns	ns	ns	ns	*	*	*	*	*	*	*	*	*	*	*
C5	0.494	0.498	0.521	0.522	0.127	0.116	0.125	0.095	0.088	0.001	0.000	0.020	-	ns	ns	ns	ns	ns	*	*	*	*	*	*	*	*	*	*
C6	0.500	0.497	0.502	0.505	0.166	0.131	0.139	0.129	0.097	0.022	0.015	0.011	0.039	-	ns	ns	ns	ns	*	*	*	*	*	*	*	*	*	*
C8	0.490	0.490	0.494	0.495	0.160	0.121	0.108	0.125	0.121	0.021	0.020	0.035	0.019	0.014	-	ns	ns	ns	*	*	*	*	*	*	*	*	*	*
C10	0.486	0.492	0.508	0.504	0.134	0.120	0.097	0.096	0.113	0.004	0.003	0.029	0.015	0.037	0.017	-	ns	ns	*	*	*	*	*	*	*	*	*	*
C11	0.506	0.506	0.499	0.499	0.174	0.138	0.098	0.147	0.171	0.056	0.060	0.078	0.062	0.046	0.013	0.037	-	ns	*	*	*	*	*	*	*	*	*	*
C12	0.499	0.505	0.503	0.496	0.177	0.141	0.122	0.117	0.186	0.025	0.068	0.081	0.050	0.073	0.043	0.021	0.023	-	*	*	*	*	*	*	*	*	*	*
G1	0.416	0.414	0.433	0.440	0.167	0.155	0.233	0.166	0.383	0.286	0.309	0.289	0.292	0.299	0.309	0.304	0.347	0.329	-	*	*	*	*	*	*	*	*	*
G2	0.456	0.450	0.464	0.488	0.203	0.208	0.281	0.228	0.405	0.333	0.348	0.336	0.335	0.336	0.354	0.350	0.388	0.395	0.133	-	*	*	*	*	*	*	*	*
G3	0.359	0.357	0.338	0.356	0.093	0.047	0.108	0.072	0.332	0.209	0.255	0.232	0.237	0.230	0.229	0.236	0.243	0.242	0.111	0.177	-	ns	ns	ns	ns	ns	ns	*
G4	0.349	0.349	0.349	0.351	0.094	0.046	0.111	0.068	0.331	0.199	0.246	0.218	0.220	0.230	0.224	0.219	0.232	0.224	0.132	0.202	0.009	-	ns	ns	ns	ns	ns	*
G6	0.335	0.333	0.344	0.360	0.070	0.051	0.124	0.069	0.307	0.210	0.231	0.208	0.219	0.222	0.229	0.224	0.253	0.258	0.101	0.144	0.011	0.025	-	ns	ns	ns	ns	*
G7	0.354	0.365	0.365	0.363	0.102	0.067	0.122	0.072	0.359	0.221	0.274	0.265	0.239	0.274	0.249	0.233	0.252	0.231	0.142	0.220	0.021	0.017	0.037	-	*	ns	ns	*
G11	0.378	0.367	0.353	0.370	0.088	0.037	0.068	0.060	0.287	0.167	0.208	0.190	0.192	0.170	0.171	0.192	0.182	0.200	0.170	0.229	0.042	0.064	0.067	0.081	-	ns	ns	*
G12	0.360	0.355	0.360	0.371	0.072	0.032	0.081	0.042	0.266	0.155	0.180	0.171	0.171	0.161	0.170	0.175	0.183	0.201	0.152	0.198	0.053	0.071	0.058	0.083	0.008	-	ns	ns
G16a	0.371	0.375	0.349	0.350	0.084	0.034	0.079	0.061	0.333	0.186	0.244	0.236	0.220	0.224	0.205	0.199	0.200	0.184	0.164	0.239	0.030	0.015	0.061	0.030	0.045	0.059	-	*
G18	0.391	0.390	0.392	0.410	0.066	0.060	0.113	0.069	0.258	0.173	0.177	0.185	0.172	0.178	0.192	0.179	0.210	0.231	0.199	0.213	0.114	0.116	0.085	0.139	0.094	0.072	0.121	-

Table 16. Results of Isolation By Distance (IBD) analyses for five fish taxa performed at the individual-level and conducted within river systems in which all species were sampled: the hydrologically isolated Finke River, and the hydrologically-connected Neales and Georgina-Diamantina rivers. Regressions were performed using river channel distance (km) and Rousset's *a* genetic distance, with a sample size of *n* and across a maximum distance of *D* (km). Results include the slope (x10^{-5} *a*/km) and intercept (*a*) of the regression (with 95% confidence intervals) and outcome of significance tests (*P*: p-value arising from statistical test of IBD – values below 0.05 indicate significant IBD).

Taxa	*n*	D	IBD	Slope	95% CI	Intercept	95% CI
Hydrologically Isolated Finke River							
Goby	67	430	<0.001	177.332	103.534 – 273.320	-0.034	-0.138 – 0.072
Hardyhead	70	178	<0.001	9.843	1.217 – 19.036	0.110	0.083 – 0.141
Grunter	52	314	0.029	13.424	-9.160 – 29.856	0.089	0.061 – 0.118
Perch	57	484	0.026	14.786	6.221 – 24.510	0.137	0.113 – 0.162
Herring	35	466	0.002	18.599	7.906 – 31.430	0.081	0.051 – 0.113
Hydrologically-Connected Neales and Georgina-Diamantina rivers							
Goby	66	598	<0.001	67.880	53.942 – 85.014	0.187	0.151 – 0.224
Hardyhead	34	755	<0.001	8.837	4.800 – 13.394	0.107	0.080 – 0.135
Grunter	31	1261	0.038	1.946	-0.135 – 4.240	0.266	0.232 – 0.300
Perch	63	2040	<0.001	8.056	6.990 – 9.208	0.153	0.135 – 0.170
Herring	115	2137	<0.001	2.636	1.985 – 3.345	0.008	0.067 – 0.093

Appendix 2.5 – Contemporary Gene Flow Levels

Levels of contemporary gene flow were calculated using BayesAss (see Chapter 5 methods) for each of five fish taxa at two scales: among rivers and within rivers (separate analysis for each river system with at least three sampled waterholes). For within-river analyses, river-scale outgroups were included if hydrological connections exist (i.e. no outgroups used for Finke River analyses, outgroups used for all other rivers). The tables below present results averaged across five replicate runs of each analysis, with 95% credible intervals. Where the proportions of residents and/or migrants in a population are significantly greater than zero (i.e. 95% CI excludes zero) they are presented in bold. For among-rivers analyses, river systems are named (Georgina-Diamantina river system abbreviated to G-D) and can be seen on Fig. 1. For within-river analyses, site codes are mapped in Fig. 1 and more details are available in Appendix A. All sites ordered from upstream to downstream.

PART A – Finke and Desert Goby

Table 1. Recent migration rates of Finke and desert goby among rivers of the Lake Eyre Basin. Values on the diagonal are the percentages of resident individuals in each river. Other values are migration rates from rivers in columns into rivers in rows. 95% credible intervals are in parentheses. Bold values are significantly greater than zero (95% CI excludes zero).

		Source Population				
		Finke	**Neales**	**South-West**	**Frome**	**G-D**
Sampled Population	**Finke**	**0.9815** (0.9639–0.9991)	0.0046 (-0.0044–0.0136)	0.0046 (-0.0044–0.0136)	0.0046 (-0.0044–0.0137)	0.0046 (-0.0044–0.0136)
	Neales	0.0139 (-0.0047–0.0325)	**0.9652** (0.9366–0.9938)	0.0069 (-0.0064–0.0203)	0.0070 (-0.0064–0.0203)	0.0070 (-0.0065–0.0205)
	S-W	0.0090 (-0.0081–0.0261)	0.0090 (-0.0081–0.0262)	**0.9639** (0.9310–0.9969)	0.0091 (-0.0081–0.0263)	0.0090 (-0.0082–0.0263)
	Frome	0.0237 (-0.0196–0.0670)	**0.2094** (0.1254–0.2935)	0.0238 (-0.0195–0.0671)	**0.6905** (0.6470–0.7340)	0.0526 (-0.0121–0.1174)
	G-D	0.0115 (-0.0103–0.0333)	**0.0808** (0.0295–0.1321)	0.0115 (-0.0103–0.0333)	0.0115 (-0.0103–0.0332)	**0.8847** (0.8278–0.9416)

Table 2. Recent migration rates of Finke goby within the Finke River. Values on the diagonal are percentages of resident individuals in each waterhole. Other values are migration rates from waterholes in columns into waterholes in rows. 95% credible intervals in parentheses. Bold values are significantly greater than zero (95% CI excludes zero).

		Source Population									
		F3a	**F3c**	**F3d**	**F3e**	**F4**	**F5**	**F6b**	**F6c**	**F7**	**F8**
Sampled Population	**F3a**	**0.6905** (0.6471–0.7338)	0.0241 (-0.0199–0.0681)	0.0238 (-0.0196–0.0672)	0.0238 (-0.0196–0.0672)	0.0420 (-0.0089–0.0931)	0.0250 (-0.0202–0.0695)	0.0240 (-0.0197–0.0673)	0.0240 (-0.0197–0.0674)	**0.1000** (0.0260 – 0.1729)	0.0240 (-0.0197–0.0674)
	F3c	0.0166 (-0.0144–0.0476)	**0.7449** (0.6953–0.7945)	0.0167 (-0.0144–0.0478)	0.0167 (-0.0144–0.0477)	0.0170 (-0.0144–0.0477)	**0.1170** 0.0616–0.1715)	0.0170 (-0.0144–0.0477)	0.0170 (-0.0144–0.0479)	0.0220 (-0.0170–0.0606)	0.0170 (-0.0145–0.0479)
	F3d	0.0303 (-0.0240–0.0846)	0.0425 (-0.0192–0.1041)	**0.6970** (0.6428–0.7512)	0.0303 (-0.0239–0.0844)	0.0300 (-0.0240–0.0846)	0.0490 (-0.0168–0.1137)	0.0300 (-0.0240–0.0846)	0.0300 (-0.0239–0.0844)	0.0300 (-0.0239–0.0846)	0.0300 (-0.0238–0.0844)
	F3e	0.0303 (-0.0240–0.0847)	0.0425 (-0.0193–0.1042)	0.0303 (-0.0239–0.0844)	**0.6970** (0.6427–0.7512)	0.0300 (-0.0239–0.0845)	0.0480 (-0.0168–0.1136)	0.0300 (-0.024–0.0848)	0.0300 (-0.024–0.0846)	0.0300 (-0.0239–0.0845)	0.0300 (-0.0239–0.0844)
	F4	0.0175 (-0.0150–0.0500)	0.0175 (-0.0150–0.0500)	0.0175 (-0.015–0.0501)	0.0175 (-0.0151–0.0501)	**0.7010** (0.6617–0.7411)	0.018 (-0.0151–0.0503)	0.018 (-0.0152–0.0502)	0.025 (-0.0181–0.068)	**0.151** (0.0747–0.2273)	0.0180 (-0.0151–0.0501)
	F5	0.0167 (-0.0143–0.0477)	**0.0831** (0.0360–0.1302)	0.0167 (-0.0144–0.0478)	0.0167 (-0.0145–0.0479)	0.018 (-0.0148–0.0499)	**0.776** (0.7187–0.8334)	0.017 (-0.0145–0.0479)	0.019 (-0.0148–0.0535)	0.021 (-0.0159–0.0569)	0.017 (-0.0143–0.0478)
	F6b	0.0257 (-0.0209–0.0722)	0.0257 (-0.0210–0.0723)	0.0256 (-0.0208–0.0721)	0.0256 (-0.0209–0.0722)	0.041 (-0.0125–0.0945)	0.026 (-0.021–0.0723)	**0.6920** 0.6457–0.7389)	0.0260 (-0.0209–0.0722)	**0.0870** (0.0132–0.1612)	0.0260 (-0.0209–0.0720)
	F6c	0.0175 (-0.0150–0.0500)	0.0176 (-0.0152–0.0503)	0.0175 (-0.0151–0.0501)	0.0175 (-0.0151–0.0502)	0.0280 (-0.0100–0.0659)	0.0180 (-0.0151–0.0501)	0.0180 (-0.0151–0.0501)	**0.6840** (0.6516–0.7169)	**0.1650** (0.0921–0.2382)	0.0180 (-0.0151–0.0501)
	F7	0.0166 (-0.0144–0.0477)	0.0167 (-0.0143–0.0478)	0.0166 (-0.0144–0.0476)	0.0170 (-0.0144–0.0478)	**0.0500** (0.0103–0.0887)	0.0170 (-0.0144–0.0478)	0.0170 (-0.0144–0.0477)	**0.04** (0.0012–0.0789)	**0.7940** (0.7329–0.8547)	0.0170 (-0.0144–0.0478)
	F8	0.0167 (-0.0143–0.0477)	0.0167 (-0.0144–0.0478)	0.0167 (-0.0144–0.0477)	0.017 (-0.0144–0.0477)	0.0330 (-0.0048–0.071)	0.0170 (-0.0145–0.0479)	0.0170 (-0.0144–0.0476)	0.0330 (-0.0048–0.0713)	**0.1500** (0.0813–0.2195)	**0.6830** (0.6523–0.7143)

Table 3. Recent migration rates of desert goby within the Neales River. Values on the diagonal are percentages of resident individuals. Other values are migration rates from sites in columns into sites in rows. 95% credible intervals in parentheses. Bold values are significantly greater than zero (95% CI excludes zero).

		Source Population									
		N1	**N2a**	**N2b**	**N3**	**N4**	**N5**	**N6**	**S-W**	**Frome**	**G-D**
Sampled Population	**N1**	**0.6889** *(0.6481–0.7297)*	0.0222 *(-0.0186–0.0630)*	0.0222 *(-0.0186–0.0631)*	0.0222 *(-0.0184–0.0629)*	0.0222 *(-0.0185–0.0629)*	**0.1333** *(0.0534–0.2132)*	0.0222 *(-0.0185–0.0630)*	0.0222 *(-0.0185–0.0629)*	0.0223 *(-0.0185–0.0630)*	0.0222 *(-0.0185–0.0629)*
	N2a	0.0208 *(-0.0176–0.0592)*	**0.7405** *(0.6733–0.8078)*	0.0208 *(-0.0176–0.0592)*	0.0208 *(-0.0175–0.0592)*	0.0209 *(-0.0175–0.0593)*	**0.0928** *(0.0205–0.1651)*	0.0208 *(-0.0176–0.0592)*	0.0208 *(-0.0175–0.0591)*	0.0208 *(-0.0175–0.0592)*	0.0208 *(-0.0175–0.0592)*
	N2b	0.0222 *(-0.0185–0.0630)*	0.0222 *(-0.0185–0.0628)*	**0.7067** *(0.6541–0.7592)*	0.0222 *(-0.0184–0.0629)*	0.0222 *(-0.0185–0.0628)*	**0.1158** *(0.0381–0.1935)*	0.0222 *(-0.0185–0.0629)*	0.0222 *(-0.0185–0.0629)*	0.0222 *(-0.0185–0.0629)*	0.0222 *(-0.0185–0.0629)*
	N3	0.0222 *(-0.0186–0.0629)*	0.0222 *(-0.0184–0.0629)*	0.0223 *(-0.0185–0.0630)*	**0.7555** *(0.6833–0.8277)*	0.0222 *(-0.0185–0.0629)*	**0.0666** *(0.0014–0.1318)*	0.0223 *(-0.0186–0.0631)*	0.0222 *(-0.0185–0.0630)*	0.0223 *(-0.0185–0.0630)*	0.0222 *(-0.0186–0.0630)*
	N4	0.0208 *(-0.0174–0.0591)*	0.0208 *(-0.0174–0.0590)*	0.0208 *(-0.0175–0.0592)*	0.0208 *(-0.0175–0.0591)*	**0.6875** *(0.6492–0.7258)*	**0.1461** *(0.0674–0.2248)*	0.0208 *(-0.0175–0.0592)*	0.0208 *(-0.0175–0.0590)*	0.0208 *(-0.0175–0.0592)*	0.0208 *(-0.0175–0.0592)*
	N5	0.0209 *(-0.0176–0.0593)*	0.0208 *(-0.0175–0.0590)*	0.0208 *(-0.0176–0.0593)*	0.0208 *(-0.0175–0.0592)*	0.0209 *(-0.0175–0.0593)*	**0.8125** *(0.7338–0.8912)*	0.0208 *(-0.0176–0.0592)*	0.0209 *(-0.0175–0.0592)*	0.0208 *(-0.0174–0.0589)*	0.0208 *(-0.0175–0.0591)*
	N6	0.0167 *(-0.0143–0.0477)*	0.0166 *(-0.0144–0.0476)*	0.0167 *(-0.0143–0.0477)*	0.0166 *(-0.0144–0.0477)*	0.0167 *(-0.0144–0.0477)*	**0.1836** *(0.1126–0.2546)*	**0.6833** *(0.6523–0.7143)*	0.0166 *(-0.0144–0.0476)*	0.0166 *(-0.0144–0.0476)*	0.0166 *(-0.0144–0.0477)*
	S-W	0.0079 *(-0.0073–0.023)*	0.0079 *(-0.0073–0.0231)*	0.0079 *(-0.0073–0.0232)*	0.0079 *(-0.0073–0.0231)*	0.0079 *(-0.0074–0.0232)*	0.0080 *(-0.0072–0.0232)*	0.0080 *(-0.0072–0.0232)*	**0.9285** *(0.8876–0.9694)*	0.0080 *(-0.0073–0.0232)*	0.0080 *(-0.0073–0.0232)*
	Frome	0.0175 *(-0.0151–0.0501)*	0.0175 *(-0.015–0.05)*	0.0176 *(-0.015–0.0501)*	0.0176 *(-0.0151–0.0503)*	0.0175 *(-0.0151–0.0502)*	**0.1289** *(0.0575–0.2003)*	0.0175 *(-0.0151–0.0502)*	0.0175 *(-0.0151–0.0502)*	**0.7308** *(0.6729–0.7886)*	0.0176 *(-0.015–0.0502)*
	G-D	0.0098 *(-0.0089–0.0284)*	0.0097 *(-0.0088–0.0283)*	0.0098 *(-0.009–0.0285)*	0.0098 *(-0.0088–0.0284)*	0.0098 *(-0.0088–0.0285)*	**0.0687** *(0.0241–0.1133)*	0.0098 *(-0.0089–0.0285)*	0.0098 *(-0.0088–0.0284)*	0.0098 *(-0.0089–0.0285)*	**0.8530** *(0.7983–0.9078)*

Table 4. Recent migration rates of desert goby within the South-West Creeks. Values on the diagonal are percentages of resident individuals. Other values are migration rates from sites in columns into sites in rows. 95% credible intervals in parentheses. Bold values are significantly greater than zero (95% CI excludes zero).

		Source Population						
		S1	**S2**	**S3**	**S4**	**Neales**	**Frome**	**G-D**
Sampled Population	**S1**	**0.6900** (0.6471–0.7339)	0.0238 (-0.0196–0.0672)	0.0238 (-0.0196–0.0672)	**0.1910** (0.1072–0.2741)	0.0238 (-0.0195–0.0671)	0.0238 (-0.0197–0.0673)	0.0238 (-0.0197–0.0672)
	S2	0.0260 (-0.0208–0.072)	**0.6923** (0.6458–0.7387)	0.0257 (-0.0209–0.0723)	**0.1800** (0.0926–0.2664)	0.0256 (-0.0209–0.0722)	0.0257 (-0.0209–0.0722)	0.0256 (-0.0209–0.0722)
	S3	0.0210 (-0.0175–0.0591)	0.0208 (-0.0175–0.0591)	**0.6875** (0.6491–0.7259)	**0.2080** (0.1317–0.2851)	0.0208 (-0.0175–0.0592)	0.0208 (-0.0175–0.0591)	0.0208 (-0.0175–0.0592)
	S4	0.0200 (-0.0166–0.0559)	0.0196 (-0.0166–0.0558)	0.0196 (-0.0166–0.0559)	**0.8820** (0.8087–0.956)	0.0196 (-0.0168–0.0559)	0.0196 (-0.0167–0.0559)	0.0196 (-0.0166–0.0558)
	Neales	0.0070 (-0.0061–0.0194)	0.0070 (-0.0060–0.0194)	0.007 (-0.0061–0.0195)	0.0070 (-0.0062–0.0196)	**0.9520** (0.9199–0.9841)	0.015 (-0.004–0.0333)	0.007 (-0.006–0.0194)
	Frome	0.0210 (-0.0176–0.0593)	0.0208 (-0.0175–0.0591)	0.0208 (-0.0175–0.0592)	0.0210 (-0.0176–0.0593)	**0.1708** (0.0916–0.2499)	**0.7250** (0.665–0.785)	0.0209 (-0.0175–0.0593)
	G-D	0.0110 (-0.0097–0.0312)	0.0108 (-0.0096–0.0311)	0.0107 (-0.0096–0.0311)	0.0110 (-0.0097–0.0311)	**0.0750** (0.0270–0.1236)	0.0107 (-0.0097–0.0312)	**0.8710** (0.8147–0.9272)

PART B – Finke and Lake Eyre Hardyhead

Table 5. Recent migration rates of Finke and Lake Eyre hardyhead among rivers of the Lake Eyre Basin. Values on the diagonal are the percentages of resident individuals in each river. Other values are migration rates from rivers in columns into rivers in rows. 95% credible intervals are in parentheses. Bold values are significantly greater than zero (95% CI excludes zero).

		Source Population				
		Finke	**Neales**	**South-West**	**Frome**	**G-D**
Sampled Population	**Finke**	**0.9822** (0.9654–0.9991)	0.0045 (-0.0041–0.013)	0.0044 (-0.0042–0.013)	0.0044 (-0.0042–0.0131)	0.0044 (-0.0042–0.013)
	Neales	0.0115 (-0.0103–0.0334)	**0.9540** (0.9128–0.9952)	0.0115 (-0.0103–0.0333)	0.0115 (-0.0102–0.0332)	0.0115 (-0.0103–0.0333)
	S-W	0.0085 (-0.0078–0.0248)	0.0086 (-0.0077–0.0249)	**0.9658** (0.9344–0.9972)	0.0085 (-0.0078–0.0249)	0.0085 (-0.0078–0.0249)
	Frome	0.0417 (-0.0306–0.1139)	**0.1669** (0.058–0.2758)	0.0416 (-0.0305–0.1138)	**0.7083** (0.6365–0.7801)	0.0415 (-0.0305–0.1135)
	G-D	0.0223 (-0.0185–0.063)	**0.2442** (0.1717–0.3167)	0.0223 (-0.0185–0.0631)	0.0223 (-0.0186–0.0633)	**0.6889** (0.648–0.7299)

Table 6. Recent migration rates of Finke hardyhead within the Finke River. Values on the diagonal are percentages of resident individuals in each waterhole. Other values are migration rates from waterholes in columns into waterholes in rows. 95% credible intervals in parentheses. Bold values are significantly greater than zero (95% CI excludes zero).

		Source Population					
		F3d	**F3e**	**F4**	**F5**	**F6a**	**F6b**
Sampled Population	**F3d**	**0.6833** (0.6521–0.7145)	0.0166 (-0.0144–0.0476)	0.0166 (-0.0144–0.0476)	0.0166 (-0.0144–0.0476)	0.0167 (-0.0143–0.0477)	**0.2502** (0.1885–0.3119)
	F3e	0.0303 (-0.024–0.0846)	**0.6969** (0.6428–0.751)	0.0303 (-0.0238–0.0844)	0.0303 (-0.024–0.0846)	0.0303 (-0.0238–0.0844)	**0.1819** (0.088–0.2758)
	F4	0.0208 (-0.0176–0.0592)	0.0209 (-0.0175–0.0593)	**0.6875** (0.6491–0.7259)	0.0208 (-0.0176–0.0592)	0.0209 (-0.0175–0.0593)	**0.2290** (0.1555–0.3025)
	F5	0.0208 (-0.0176–0.0592)	0.0208 (-0.0174–0.059)	0.0208 (-0.0176–0.0592)	**0.6875** (0.6491–0.7259)	0.0208 (-0.0174–0.059)	**0.2292** (0.1557–0.3027)
	F6a	0.0196 (-0.0167–0.0559)	0.0196 (-0.0167–0.0559)	0.0196 (-0.0167–0.0559)	0.0196 (-0.0167–0.0559)	**0.6863** (0.65–0.7226)	**0.2353** (0.1653–0.3053)
	F6b	0.0130 (-0.0115–0.0371)	0.0130 (-0.0113–0.0369)	0.0130 (-0.0113–0.0369)	0.0130 (-0.0113–0.0369)	0.0130 (-0.0113–0.0369)	**0.9360** (0.8865–0.9853)

Table 7. Recent migration rates of Lake Eyre hardyhead within the Neales River. Values on the diagonal are percentages of resident individuals. Other values are migration rates from sites in columns into sites in rows. 95% credible intervals in parentheses. Bold values are significantly greater than zero (95% CI excludes zero).

		Source Population					
		N1	**N2b**	**N3**	**South-West**	**Frome**	**G-D**
Sampled Population	**N1**	**0.6949** (0.6439–0.7459)	**0.1939** (0.1041–0.2837)	0.0278 (-0.0224–0.0780)	0.0278 (-0.0222–0.0778)	0.0278 (-0.0224–0.078)	0.0278 (-0.0224–0.078)
	N2b	0.0208 (-0.0176–0.0592)	**0.8958** (0.8223–0.9693)	0.0209 (-0.0175–0.0593)	0.0208 (-0.0176–0.0592)	0.0208 (-0.0174–0.059)	0.0208 (-0.0176–0.0592)
	N3	0.0238 (-0.0197–0.0673)	**0.2144** (0.1336–0.2952)	**0.6905** (0.6470–0.7340)	0.0238 (-0.0197–0.0673)	0.0238 (-0.0195–0.0671)	0.0238 (-0.0195–0.0671)
	S-W	0.0083 (-0.0076–0.0242)	0.0083 (-0.0076–0.0242)	0.0083 (-0.0076–0.0242)	**0.9583** (0.9246–0.992)	0.0083 (-0.0076–0.0242)	0.0083 (-0.0076–0.0242)
	Frome	0.0371 (-0.028–0.1022)	**0.1481** (0.0454–0.2508)	0.0370 (-0.0279–0.1019)	0.0370 (-0.0279–0.1019)	**0.7037** (0.6386–0.7688)	0.0370 (-0.0279–0.1019)
	G-D	0.0210 (-0.0176–0.0592)	**0.2110** (0.1339–0.2875)	0.0210 (-0.0174–0.0590)	0.0210 (-0.0174–0.0590)	0.0210 (-0.0176–0.0592)	**0.7060** (0.6542–0.7576)

Table 8. Recent migration rates of Lake Eyre hardyhead within the South-West Creeks. Values on the diagonal are percentages of resident individuals. Other values are migration rates from sites in columns into sites in rows. 95% credible intervals in parentheses. Bold values are significantly greater than zero (95% CI excludes zero).

		Source Population						
		S1	**S2**	**S3**	**S4**	**Neales**	**Frome**	**G-D**
Sampled Population	**S1**	**0.7020** (0.6552–0.7488)	0.0196 (-0.0167–0.0559)	0.0196 (-0.0167–0.0559)	0.0196 (-0.0167–0.0559)	**0.2000** (0.1247–0.2753)	0.0196 (-0.0167–0.0559)	0.0196 (-0.0197–0.0672)
	S2	0.0238 (-0.0197–0.0673)	**0.6905** (0.6470–0.7340)	**0.1153** (0.0369–0.1937)	0.0238 (-0.0197–0.0673)	0.0239 (-0.0196–0.0674)	**0.0988** (0.0239–0.1737)	0.0238 (-0.0209–0.0722)
	S3	0.0196 (-0.0167–0.0559)	0.0196 (-0.0167–0.0559)	**0.8006** (0.7387–0.8625)	0.0196 (-0.0167–0.0559)	0.0196 (-0.0167–0.0559)	**0.1015** (0.0439–0.1591)	0.0196 (-0.0175–0.0592)
	S4	0.0239 (-0.0196–0.0674)	0.0238 (-0.0197–0.0673)	0.0317 (-0.0179–0.0813)	**0.7393** (0.6629–0.8157)	0.0238 (-0.0197–0.0673)	**0.1337** (0.0463–0.2211)	0.0238 (-0.0166–0.0558)
	Neales	0.0108 (-0.0096–0.0312)	0.0108 (-0.0096–0.0312)	0.0108 (-0.0096–0.0312)	0.0108 (-0.0096–0.0312)	**0.9355** (0.8898–0.9812)	0.0108 (-0.0096–0.0312)	0.0107 (-0.006–0.0194)
	Frome	0.0196 (-0.0167–0.0559)	0.0196 (-0.0167–0.0559)	**0.1219** (0.0555–0.1883)	0.0197 (-0.0168–0.0562)	0.0196 (-0.0165–0.0557)	**0.7800** (0.7116–0.8484)	0.0196 (-0.0175–0.0593)
	G-D	0.0334 (-0.0258–0.0926)	0.0333 (-0.0257–0.0923)	0.0333 (-0.0259–0.0925)	0.0333 (-0.0257–0.0923)	**0.1334** (0.037–0.2298)	0.0333 (-0.0257–0.0923)	**0.7000** (0.8147–\0.9272)

PART C – Barred Grunter

Table 9. Recent migration rates of barred grunter among rivers of the Lake Eyre Basin. Values on the diagonal are the percentages of resident individuals in each river. Other values are migration rates from rivers in columns into rivers in rows. 95% credible intervals are in parentheses. Bold values are significantly greater than zero (95% CI excludes zero).

		Source Population		
		Finke	**Neales**	**G-D**
Sampled Population	**Finke**	**0.9879** (0.9715–1.0042)	0.0061 (-0.0056–0.0178)	0.0060 (-0.0056–0.0177)
	Neales	0.0159 (-0.0139–0.0456)	**0.9683** (0.9273–1.0092)	0.0158 (-0.0138–0.0455)
	G-D	0.0209 (-0.0176–0.0593)	0.0208 (-0.0177–0.0594)	**0.9583** (0.9058–1.0108)

Table 10. Recent migration rates of barred grunter within the Finke River. Values on the diagonal are percentages of resident individuals in each waterhole. Other values are migration rates from waterholes in columns into waterholes in rows. 95% credible intervals in parentheses. Bold values are significantly greater than zero (95% CI excludes zero).

		Source Population							
		F1	**F3b**	**F3d**	**F3e**	**F4**	**F5**	**F6a**	**F7**
Sampled Population	**F1**	**0.8704** (0.7973–0.9435)	0.0185 (-0.0158–0.0528)	0.0185 (-0.0178–0.0548)	0.0185 (-0.0158–0.0528)	0.0185 (-0.0158–0.0528)	0.0185 (-0.0158–0.0528)	0.0185 (-0.0158–0.0528)	0.0185 (-0.0158–0.0528)
	F3b	**0.0911** (0.007–0.1752)	**0.7273** (0.6546–0.8000)	0.0303 (-0.0291–0.0897)	0.0303 (-0.0238–0.0844)	0.0303 (-0.0238–0.0844)	0.0303 (-0.0240–0.0846)	0.0302 (-0.0239–0.0843)	0.0303 (-0.024–0.0846)
	F3d	**0.1874** (0.1086–0.2662)	0.0208 (-0.0176–0.0592)	**0.6875** (0.6465–0.7285)	0.0209 (-0.0175–0.0593)	0.0209 (-0.0175–0.0593)	0.0208 (-0.0176–0.0592)	0.0208 (-0.0176–0.0592)	0.0208 (-0.0176–0.0592)
	F3e	**0.1778** (0.0963–0.2593)	0.0222 (-0.0186–0.0630)	0.0222 (-1.3280–1.3724)	**0.6889** (0.6481–0.7297)	0.0222 (-0.0186–0.0630)	0.0222 (-0.0186–0.0630)	0.0222 (-0.0186–0.0630)	0.0222 (-0.0186–0.0630)
	F4	**0.1073** (0.0169–0.1977)	0.0302 (-0.0237–0.0841)	0.0304 (-0.029–0.0898)	0.0303 (-0.0240–0.0846)	**0.7110** (0.6442–0.7778)	0.0302 (-0.0237–0.0841)	0.0303 (-0.0240–0.0846)	0.0304 (-0.0241–0.0849)
	F5	**0.1960** (0.1203–0.2717)	0.0196 (-0.0165–0.0557)	0.0196 (-0.0188–0.0580)	0.0196 (-0.0167–0.0559)	0.0196 (-0.0167–0.0559)	**0.6863** (0.6500–0.7226)	0.0196 (-0.0167–0.0559)	0.0196 (-0.0167–0.0559)
	F6a	**0.2038** (0.1307–0.2769)	0.0185 (-0.0158–0.0528)	0.0185 (-0.0178–0.0548)	0.0185 (-0.0158–0.0528)	0.0185 (-0.0158–0.0528)	0.0185 (-0.0158–0.0528)	**0.6852** (0.6509–0.7195)	0.0185 (-0.0158–0.0528)
	F7	**0.1000** (0.0098–0.1902)	0.0332 (-0.0256–0.092)	0.0334 (-0.0321–0.0989)	0.0334 (-0.0258–0.0926)	0.0333 (-0.0257–0.0923)	0.0333 (-0.0257–0.0923)	0.0333 (-0.0259–0.0925)	**0.7000** (0.6408–0.7592)

Table 11. Recent migration rates of barred grunter within the Neales River. Values on the diagonal are percentages of resident individuals. Other values are migration rates from sites in columns into sites in rows. 95% credible intervals in parentheses. Bold values are significantly greater than zero (95% CI excludes zero).

		Source Population			
		N2b	**N3**	**N4**	**G-D**
Sampled Population	**N2b**	**0.9412** (0.8824–1.0000)	0.0196 (-0.0167–0.0559)	0.0196 (-0.0167–0.0559)	0.0196 (-0.0167–0.0559)
	N3	0.0371 (-0.0280–0.1022)	**0.8300** (0.7257–0.9343)	**0.0958** (0.0013–0.1902)	0.0371 (-0.0280–0.1022)
	N4	0.0257 (-0.0209–0.0723)	0.0256 (-0.0210–0.0722)	**0.9230** (0.8495–0.9965)	0.0257 (-0.0209–0.0723)
	G-D	0.0417 (-0.0304–0.1138)	0.0417 (-0.0302–0.1136)	**0.2082** (0.1028–0.3136)	**0.7084** (0.6363–0.7805)

PART D – Spangled Perch

Table 12. Recent migration rates of spangled perch among rivers of the Lake Eyre Basin. Values on the diagonal are the percentages of resident individuals in each river. Other values are migration rates from rivers in columns into rivers in rows. 95% credible intervals are in parentheses. Bold values are significantly greater than zero (95% CI excludes zero).

		Source Population			
		Finke	**Neales**	**Cooper**	**G-D**
Sampled Population	**Finke**	**0.9836** (0.9656–1.0016)	0.0055 (-0.0051–0.01608)	0.0055 (-0.0051–0.0161)	0.0055 (-0.0051–0.0161)
	Neales	0.0139 (-0.0122–0.0399)	**0.9584** (0.9153–1.0015)	0.0139 (-0.0122–0.0399)	0.0139 (-0.0122–0.0399)
	Cooper	0.0101 (-0.0091–0.0293)	0.0101 (-0.0091–0.0293)	**0.9697** (0.9376–1.0018)	0.0101 (-0.0091–0.0293)
	G-D	0.0071 (-0.0066–0.0208)	0.0175 (-0.0039–0.0389)	0.0071 (-0.0064–0.0206)	**0.9683** (0.9403–0.9963)

Table 13. Recent migration rates of spangled perch within the Neales River. Values on the diagonal are percentages of resident individuals. Other values are migration rates from sites in columns into sites in rows. 95% credible intervals in parentheses. Bold values are significantly greater than zero (95% CI excludes zero).

		Source Population					
		N1	**N2b**	**N3**	**N4**	**Cooper**	**G-D**
Sampled Population	**N1**	**0.7528** (0.6524–0.8532)	0.0476 (-0.0333–0.1285)	0.0475 (-0.0333–0.1283)	0.0562 (-0.0296–0.142)	0.0477 (-0.0332–0.1286)	0.0481 (-0.0336–0.1298)
	N2b	0.0476 (-0.0333–0.1285)	**0.7143** (0.6334–0.7952)	0.0477 (-0.0334–0.1288)	0.0952 (-0.0093–0.1997)	0.0476 (-0.0333–0.1285)	0.0476 (-0.0333–0.1285)
	N3	0.0238 (-0.0197–0.0673)	0.0239 (-0.0196–0.0674)	**0.7358** (0.6666–0.805)	**0.1689** (0.084–0.2538)	0.0238 (-0.0197–0.0673)	0.0238 (-0.0195–0.0671)
	N4	0.0208 (-0.0174–0.059)	0.0209 (-0.0175–0.0593)	0.0208 (-0.0176–0.0592)	**0.8958** (0.8223–0.9693)	0.0208 (-0.0176–0.0592)	0.0208 (-0.0176–0.0592)
	Cooper	0.0095 (-0.0085–0.0275)	0.0095 (-0.0085–0.0275)	0.0095 (-0.0087–0.0277)	0.0095 (-0.0087–0.0277)	**0.9523** (0.9141–0.9905)	0.0095 (-0.0087–0.0277)
	G-D	0.0068 (-0.0063–0.0199)	0.0068 (-0.0063–0.0199)	0.0068 (-0.0061–0.0197)	0.0170 (-0.0036–0.0376)	0.0074 (-0.0065–0.0213)	**0.9553** (0.9235–0.9871)

Table 14. Recent migration rates of spangled perch within the Finke River. Values on the diagonal are percentages of resident individuals in each waterhole. Other values are migration rates from waterholes in columns into waterholes in rows. 95% credible intervals in parentheses. Bold values are significantly greater than zero (95% CI excludes zero).

		Source Population									
		F1	**F2**	**F3a**	**F3b**	**F3d**	**F3e**	**F4**	**F5**	**F6c**	**F9**
Sampled Population	**F1**	**0.7290** *(0.6606–0.7974)*	0.0238 *(-0.0197–0.0673)*	0.0238 *(-0.0195–0.0671)*	0.0237 *(-0.0196–0.067)*	0.0238 *(-0.0197–0.0673)*	0.0492 *(-0.0118–0.1102)*	0.0315 *(-0.0171–0.0801)*	0.0238 *(-0.0195–0.0671)*	0.0238 *(-0.0197–0.0673)*	0.0238 *(-0.0197–0.0673)*
	F2	0.0208 *(-0.0174–0.0590)*	**0.6875** *(0.6493–0.7257)*	0.0208 *(-0.0176–0.0592)*	0.0208 *(-0.0174–0.0590)*	0.0208 *(-0.0176–0.0592)*	**0.1043** *(0.0355–0.1731)*	0.0418 *(-0.0043–0.0879)*	0.0208 *(-0.0174–0.0590)*	0.0208 *(-0.0174–0.0590)*	0.0209 *(-0.0175–0.0593)*
	F3a	0.0185 *(-0.0158–0.0528)*	0.0185 *(-0.0158–0.0528)*	**0.7107** *(0.6576–0.7638)*	0.0185 *(-0.0158–0.0528)*	0.0185 *(-0.0158–0.0528)*	**0.1025** *(0.0368–0.1682)*	0.0387 *(-0.0040–0.0814)*	0.0185 *(-0.0160–0.0530)*	0.0185 *(-0.0158–0.0528)*	0.0185 *(-0.0158–0.0528)*
	F3b	0.0238 *(-0.0195–0.0671)*	0.0238 *(-0.0197–0.0673)*	0.0238 *(-0.0197–0.0673)*	**0.7113** *(0.6531–0.7695)*	0.0238 *(-0.0197–0.0673)*	0.0649 *(-0.0008–0.1306)*	0.0333 *(-0.0153–0.0819)*	0.0238 *(-0.0197–0.0673)*	0.0239 *(-0.0196–0.0674)*	0.0238 *(-0.0197–0.0673)*
	F3d	0.0159 *(-0.0137–0.0455)*	0.0159 *(-0.0137–0.0455)*	0.0158 *(-0.0140–0.0456)*	0.0159 *(-0.0137–0.0455)*	**0.6825** *(0.6529–0.7121)*	**0.1430** *(0.0813–0.2047)*	**0.0475** *(0.0099–0.0851)*	0.0158 *(-0.0138–0.0454)*	0.0159 *(-0.0137–0.0455)*	0.0159 *(-0.0137–0.0455)*
	F3e	0.0158 *(-0.014–0.0456)*	0.0159 *(-0.0137–0.0455)*	0.0159 *(-0.0139–0.0457)*	0.0159 *(-0.0137–0.0455)*	0.0159 *(-0.0137–0.0455)*	**0.8095** *(0.7480–0.8710)*	**0.0476** *(0.0098–0.0854)*	0.0159 *(-0.0137–0.0455)*	0.0159 *(-0.0139–0.0457)*	0.0158 *(-0.0138–0.0454)*
	F4	0.0158 *(-0.0138–0.0454)*	0.0159 *(-0.0137–0.0455)*	0.0159 *(-0.0137–0.0455)*	0.0159 *(-0.0139–0.0457)*	0.0158 *(-0.0138–0.0454)*	**0.1428** *(0.0809–0.2047)*	**0.7243** *(0.6767–0.7519)*	0.0159 *(-0.0137–0.0455)*	0.0159 *(-0.0139–0.0457)*	0.0159 *(-0.0137–0.0455)*
	F5	0.0278 *(-0.0224–0.0780)*	0.0278 *(-0.0222–0.0778)*	0.0278 *(-0.0224–0.0780)*	0.0278 *(-0.0222–0.0778)*	0.0277 *(-0.0223–0.0777)*	0.0500 *(-0.0141–0.1141)*	0.0333 *(-0.0204–0.0870)*	**0.6944** *(0.6444–0.7444)*	0.0278 *(-0.0222–0.0778)*	0.0278 *(-0.0222–0.0778)*
	F6c	0.0208 *(-0.0176–0.0592)*	0.0208 *(-0.0176–0.0592)*	0.0208 *(-0.0174–0.0590)*	0.0208 *(-0.0176–0.0592)*	0.0208 *(-0.0174–0.0590)*	**0.1044** *(0.0352–0.1736)*	0.0416 *(-0.0045–0.0877)*	0.0208 *(-0.0174–0.0590)*	**0.6875** *(0.6491–0.7259)*	0.0208 *(-0.0174–0.0590)*
	F9	0.0257 *(-0.0208–0.0722)*	0.0256 *(-0.0209–0.0721)*	0.0257 *(-0.0208–0.0722)*	0.0256 *(-0.0210–0.0722)*	0.0257 *(-0.0208–0.0722)*	0.0502 *(-0.0119–0.1123)*	0.0327 *(-0.0188–0.0842)*	0.0257 *(-0.0209–0.0723)*	0.0256 *(-0.0209–0.0721)*	**0.7119** *(0.6507–0.7731)*

Table 15. Recent migration rates of spangled perch within Cooper Creek. Values on the diagonal are percentages of resident individuals. Other values are migration rates from sites in columns into sites in rows. 95% credible intervals in parentheses. Bold values are significantly greater than zero (95% CI excludes zero).

		Source Population								
		C1	**C2**	**C3**	**C5**	**C6**	**C7**	**C9**	**G-D**	**Neales**
Sampled Population	**C1**	**0.6904** (0.6469–0.7339)	0.0476 (-0.0038–0.0990)	0.0476 (-0.0039–0.0991)	0.0238 (-0.0197–0.0673)	0.0475 (-0.0039–0.0989)	**0.0716** (0.0122–0.1310)	0.0238 (-0.0197–0.0673)	0.0238 (-0.0195–0.0671)	0.0238 (-0.0195–0.0671)
	C2	0.0238 (-0.0197–0.0673)	**0.7143** (0.6628–0.7658)	0.0476 (-0.0038–0.0990)	0.0238 (-0.0197–0.0673)	0.0476 (-0.0039–0.0991)	**0.0715** (0.0121–0.1309)	0.0238 (-0.0197–0.0673)	0.0238 (-0.0197–0.0673)	0.0238 (-0.0197–0.0673)
	C3	0.0238 (-0.0197–0.0673)	0.0477 (-0.0038–0.0992)	**0.7143** (0.6628–0.7658)	0.0238 (-0.0197–0.0673)	0.0476 (-0.0038–0.0990)	**0.0714** (0.0118–0.1310)	0.0238 (-0.0197–0.0673)	0.0238 (-0.0197–0.0673)	0.0238 (-0.0197–0.0673)
	C5	0.0303 (-0.0240–0.0846)	0.0424 (-0.0178–0.1026)	0.0424 (-0.0178–0.1026)	**0.6970** (0.6427–0.7513)	0.0424 (-0.018–0.1028)	0.0545 (-0.0116–0.1206)	0.0303 (-0.0240–0.0846)	0.0303 (-0.0238–0.0844)	0.0303 (-0.0240–0.0846)
	C6	0.0238 (-0.0197–0.0673)	0.0476 (-0.0038–0.0990)	0.0476 (-0.0038–0.0990)	0.0238 (-0.0197–0.0673)	**0.7143** (0.6628–0.7658)	**0.0714** (0.0118–0.1310)	0.0238 (-0.0197–0.0673)	0.0238 (-0.0195–0.0671)	0.0238 (-0.0197–0.0673)
	C7	0.0238 (-0.0197–0.0673)	0.0477 (-0.0038–0.0992)	0.0476 (-0.0039–0.0991)	0.0239 (-0.0196–0.0674)	0.0476 (-0.0038–0.0990)	**0.7381** (0.6785–0.7977)	0.0238 (-0.0197–0.0673)	0.0238 (-0.0195–0.0671)	0.0239 (-0.0196–0.0674)
	C9	0.0303 (-0.0238–0.0844)	0.0425 (-0.0177–0.1027)	0.0425 (-0.0177–0.1027)	0.0303 (-0.0240–0.0846)	0.0424 (-0.0178–0.1026)	0.0545 (-0.0116–0.1206)	**0.6970** (0.6427–0.7513)	0.0303 (-0.0238–0.0844)	0.0303 (-0.0238–0.0844)
	G-D	0.0064 (-0.0059–0.0187)	0.0064 (-0.0059–0.0187)	0.0064 (-0.0059–0.0187)	0.0064 (-0.0059–0.0187)	0.0064 (-0.0059–0.0187)	0.0064 (-0.0059–0.0187)	0.0064 (-0.0059–0.0187)	**0.9383** (0.9032–0.9734)	0.0167 (-0.0033–0.0367)
	Neales	0.0114 (-0.0104–0.0332)	0.0115 (-0.0103–0.0333)	0.0115 (-0.0103–0.0333)	0.0115 (-0.0103–0.0333)	0.0115 (-0.0103–0.0333)	0.0115 (-0.0103–0.0333)	0.0115 (-0.0103–0.0333)	0.0115 (-0.0103–0.0333)	**0.9080** (0.8547–0.9613)

Table 16. Recent migration rates of spangled perch within the Georgina-Diamantina River. Values on the diagonal are percentages of resident individuals. Other values are migration rates from sites in columns into sites in rows. 95% credible intervals in parentheses. Bold values are significantly greater than zero (95% CI excludes zero).

		Source Population									
		G1	**G2**	**G5**	**G6**	**G7**	**G13**	**G16a**	**G19**	**Neales**	**Cooper**
Sampled Population	**G1**	**0.6898** *(0.6476–0.7320)*	0.0223 *(-0.0186–0.0631)*	0.0222 *(-0.0186–0.0630)*	0.0222 *(-0.0185–0.0629)*	**0.1323** *(0.0521–0.2126)*	0.0223 *(-0.0185–0.0631)*	0.0222 *(-0.0185–0.0630)*	0.022 *(-0.0185–0.0629)*	0.0222 *(-0.0185–0.0629)*	0.0222 *(-0.0185–0.0630)*
	G2	0.0222 *(-0.0186–0.0630)*	**0.7326** *(0.6278–0.8373)*	0.0222 *(-0.0185–0.0629)*	0.0223 *(-0.0185–0.0630)*	0.0896 *(-0.0198–0.1989)*	0.0222 *(-0.0185–0.0630)*	0.0222 *(-0.0185–0.0630)*	0.022 *(-0.0185–0.0629)*	0.0222 *(-0.0185–0.0630)*	0.0222 *(-0.0185–0.0630)*
	G5	0.0277 *(-0.0222–0.0777)*	0.0278 *(-0.0222–0.0778)*	**0.6945** *(0.6444–0.7446)*	0.0278 *(-0.0224–0.0779)*	**0.0834** *(0.0049–0.1618)*	0.0278 *(-0.0223–0.0778)*	0.0278 *(-0.0224–0.0780)*	0.028 *(-0.0222–0.0777)*	0.0278 *(-0.0223–0.0779)*	0.0277 *(-0.0223–0.0778)*
	G6	0.0167 *(-0.0144–0.0477)*	0.0166 *(-0.0144–0.0477)*	0.0166 *(-0.0144–0.0476)*	**0.6833** *(0.6523–0.7144)*	**0.1835** *(0.1124–0.2547)*	0.0166 *(-0.0144–0.0477)*	0.0166 *(-0.0144–0.0477)*	0.017 *(-0.0144–0.0476)*	0.0167 *(-0.0145–0.0478)*	0.0167 *(-0.0144–0.0477)*
	G7	0.0167 *(-0.0144–0.0478)*	0.0166 *(-0.0144–0.0476)*	0.0167 *(-0.0144–0.0478)*	0.0167 *(-0.0144–0.0477)*	**0.8501** *(0.7790–0.9211)*	0.0166 *(-0.0144–0.0477)*	0.0166 *(-0.0145–0.0477)*	0.017 *(-0.0144–0.0478)*	0.0167 *(-0.0145–0.0478)*	0.0166 *(-0.0144–0.0477)*
	G13	0.0303 *(-0.0239–0.0845)*	0.0303 *(-0.0238–0.0844)*	0.0303 *(-0.0238–0.0844)*	0.0303 *(-0.0239–0.0845)*	0.0303 *(-0.0239–0.0846)*	**0.6970** *(0.6427–0.7512)*	0.0606 *(-0.0121–0.1332)*	0.030 *(-0.0239–0.0844)*	0.0303 *(-0.0239–0.0845)*	0.0304 *(-0.0239–0.0846)*
	G16a	0.0209 *(-0.0175–0.0593)*	0.0208 *(-0.0176–0.0593)*	0.0208 *(-0.0175–0.0591)*	0.0208 *(-0.0174–0.0591)*	0.0417 *(-0.0107–0.0941)*	0.0209 *(-0.0176–0.0594)*	**0.7708** *(0.6974–0.8442)*	0.0210 *(-0.0176–0.0591)*	0.0416 *(-0.0107–0.0940)*	0.0208 *(-0.0175–0.0591)*
	G19	0.0238 *(-0.0194–0.0670)*	0.0238 *(-0.0197–0.0673)*	0.0238 *(-0.0196–0.0673)*	0.0238 *(-0.0196–0.0672)*	0.0562 *(-0.0095–0.1219)*	0.0238 *(-0.0196–0.0672)*	0.0238 *(-0.0197–0.0673)*	**0.7190** *(0.6513–0.7862)*	0.0585 *(-0.0083–0.1254)*	0.0238 *(-0.0196–0.0672)*
	Neales	0.0111 *(-0.0099–0.0321)*	0.0111 *(-0.0099–0.0321)*	0.0111 *(-0.0100–0.0321)*	0.0111 *(-0.0100–0.0322)*	0.0111 *(-0.0100–0.0323)*	0.0111 *(-0.0100–0.0322)*	0.0111 *(-0.0100–0.0322)*	0.0110 *(-0.0099–0.0322)*	**0.9000** *(0.8462–0.9538)*	0.0111 *(-0.0100–0.0322)*
	Cooper	0.0085 *(-0.0077–0.0248)*	0.0085 *(-0.0079–0.0249)*	0.0086 *(-0.0078–0.0249)*	0.0086 *(-0.0078–0.0249)*	0.0086 *(-0.0078–0.0249)*	0.0085 *(-0.0078–0.0249)*	0.0086 *(-0.0078–0.0250)*	0.0090 *(-0.0079–0.0249)*	0.0085 *(-0.0078–0.0248)*	**0.9231** *(0.8796–0.9666)*

PART E – Bony Herring

Table 17. Recent migration rates of bony herring among rivers of the Lake Eyre Basin. Values on the diagonal are the percentages of resident individuals in each river. Other values are migration rates from rivers in columns into rivers in rows. 95% credible intervals are in parentheses. Bold values are significantly greater than zero (95% CI excludes zero).

		Source Population				
		Finke	**Neales**	**Frome**	**Cooper**	**G-D**
Sampled Population	**Finke**	**0.9666** (0.936–0.9666)	0.0084 (-0.0076–0.0243)	0.0083 (-0.0077–0.0243)	0.0083 (-0.0076–0.0242)	0.0083 (-0.0076–0.0243)
	Neales	0.0151 (-0.0132–0.0151)	**0.6823** (0.6533–0.7114)	0.0151 (-0.0132–0.0434)	0.0151 (-0.0132–0.0435)	**0.2723** (0.2194–0.3252)
	Frome	0.0238 (-0.0196–0.0238)	0.0237 (-0.0195–0.0669)	**0.6903** (0.6472–0.7335)	0.0237 (-0.0197–0.0671)	**0.2384** (0.1626–0.3143)
	Cooper	0.0045 (-0.0042–0.0045)	0.0046 (-0.0042–0.0135)	0.0045 (-0.0042–0.0131)	**0.9789** (0.9606–0.9972)	0.0076 (-0.0038–0.0189)
	G-D	0.0032 (-0.0031–0.0032)	0.0039 (-0.0029–0.0107)	0.0032 (-0.0031–0.0095)	0.0033 (-0.003–0.0095)	**0.9864** (0.9737–0.9991)

Table 18. Recent migration rates of bony herring within the Finke River. Values on the diagonal are percentages of resident individuals in each waterhole. Other values are migration rates from waterholes in columns into waterholes in rows. 95% credible intervals in parentheses. Bold values are significantly greater than zero (95% CI excludes zero).

		Source Population					
		F3a	**F3b**	**F5**	**F6a**	**F7**	**F9**
Sampled Population	**F3a**	**0.6905** (0.647–0.7339)	0.0238 (-0.0197–0.0673)	0.0238 (-0.0196–0.0672)	**0.2144** (0.1335–0.2952)	0.0238 (-0.0196–0.0672)	0.0238 (-0.0197–0.0672)
	F3b	0.0334 (-0.0286–0.0955)	**0.6904** (0.647–0.7338)	0.0238 (-0.0196–0.0672)	**0.2048** (0.1138–0.2957)	0.0238 (-0.0196–0.0671)	0.0238 (-0.0196–0.0672)
	F5	0.0303 (-0.0239–0.0846)	0.0303 (-0.0239–0.0845)	**0.6970** (0.6427–0.7512)	**0.1818** (0.0878–0.2758)	0.0303 (-0.024–0.0846)	0.0303 (-0.024–0.0846)
	F6a	0.0209 (-0.0175–0.0592)	0.0209 (-0.0175–0.0592)	0.0208 (-0.0175–0.0592)	**0.8958** (0.8223–0.9693)	0.0208 (-0.0175–0.0591)	0.0208 (-0.0176–0.0593)
	F7	0.0370 (-0.0279–0.102)	0.037 (-0.0279–0.102)	0.037 (-0.0279–0.1019)	**0.1481** (0.0456–0.2507)	**0.7037** (0.6389–0.7686)	0.037 (-0.0278–0.1018)
	F9	0.0476 (-0.0333–0.1284)	0.0476 (-0.0332–0.1284)	0.0476 (-0.0332–0.1285)	0.0952 (-0.0091–0.1996)	0.0476 (-0.0332–0.1284)	**0.7143** (0.6335–0.7951)

Table 19. Recent migration rates of bony herring within the Neales River. Values on the diagonal are percentages of resident individuals. Other values are migration rates from sites in columns into sites in rows. 95% credible intervals in parentheses. Bold values are significantly greater than zero (95% CI excludes zero).

		Source Population					
		N2b	**N3**	**N4**	**Frome**	**Cooper**	**G-D**
Sampled Population	**N2b**	**0.6926** (0.6456–0.7396)	0.0256 (-0.0209–0.0721)	0.0257 (-0.0208–0.0722)	0.0257 (-0.0209–0.0723)	0.0256 (-0.0209–0.0721)	**0.2048** (0.1197–0.2899)
	N3	0.0416 (-0.0303–0.1135)	**0.7083** (0.6364–0.7802)	0.0416 (-0.0303–0.1135)	0.0417 (-0.0304–0.1138)	0.0417 (-0.0302–0.1136)	**0.1251** (0.0197–0.2305)
	N4	0.0239 (-0.0196–0.0674)	0.0238 (-0.0197–0.0673)	**0.6905** (0.647–0.734)	0.0238 (-0.0197–0.0673)	0.0238 (-0.0197–0.0673)	**0.2142** (0.1333–0.2951)
	Frome	0.0222 (-0.0186–0.063)	0.0222 (-0.0186–0.063)	0.0222 (-0.0186–0.063)	**0.6889** (0.6481–0.7297)	0.0223 (-0.0185–0.0631)	**0.2222** (0.1452–0.2992)
	Cooper	0.0044 (-0.004–0.0128)	0.0044 (-0.004–0.0128)	0.0044 (-0.004–0.0128)	0.0044 (-0.004–0.0128)	**0.9737** (0.9535–0.9939)	0.0088 (-0.0032–0.0208)
	G-D	0.0032 (-0.0031–0.0095)	0.0032 (-0.0031–0.0095)	0.0032 (-0.0031–0.0095)	0.0032 (-0.0031–0.0095)	0.0032 (-0.0031–0.0095)	**0.9840** (0.9703–0.9977)

Table 20. Recent migration rates of bony herring within the Cooper Creek. Values on the diagonal are percentages of resident individuals. Other values are migration rates from sites in columns into sites in rows. 95% credible intervals in parentheses. Bold values are significantly greater than zero (95% CI excludes zero).

		Source Population												
		C1	**C2**	**C3**	**C4**	**C5**	**C6**	**C8**	**C10**	**C11**	**C12**	**NR**	**FR**	**G-D**
Sampled Population	**C1**	**0.6818** (0.6536–0.7100)	0.0151 (-0.0131–0.0433)	0.0151 (-0.0131–0.0433)	0.0151 (-0.0133–0.0435)	0.0151 (-0.0131–0.0433)	0.1519 (0.0839–0.2199)	0.0151 (-0.0131–0.0433)	0.0151 (-0.0133–0.0435)	0.0151 (-0.0131–0.0433)	0.0151 (-0.0131–0.0433)	0.0151 (-0.0133–0.0435)	0.0151 (-0.0133–0.0435)	0.0151 (-0.0131–0.0433)
	C2	0.0196 (-0.0167–0.0559)	**0.6863** (0.6500–0.7226)	0.0196 (-0.0165–0.0557)	0.0196 (-0.0167–0.0559)	0.0196 (-0.0167–0.0559)	**0.0791** (0.0146–0.1436)	0.0195 (-0.0166–0.0556)	0.0196 (-0.0167–0.0559)	0.0196 (-0.0169–0.0561)	0.0386 (-0.008–0.0852)	0.0196 (-0.0167–0.0559)	0.0197 (-0.0166–0.056)	0.0197 (-0.0168–0.0562)
	C3	0.0185 (-0.0158–0.0528)	0.0186 (-0.0159–0.0531)	**0.6852** (0.6509–0.7195)	0.0185 (-0.016–0.053)	0.0186 (-0.0159–0.0531)	**0.0961** (0.0291–0.1631)	0.0185 (-0.0158–0.0528)	0.0185 (-0.0158–0.0528)	0.0185 (-0.0158–0.0528)	0.0333 (-0.0096–0.0762)	0.0186 (-0.0159–0.0531)	0.0186 (-0.0157–0.0529)	0.0185 (-0.0158–0.0528)
	C4	0.0185 (-0.016–0.0530)	0.0185 (-0.0158–0.0528)	0.0185 (-0.016–0.0530)	**0.6853** (0.6508–0.7198)	0.0185 (-0.0158–0.0528)	**0.1017** (0.0313–0.1721)	0.0185 (-0.0158–0.0528)	0.0186 (-0.0159–0.0531)	0.0185 (-0.0158–0.0528)	0.0257 (-0.0147–0.0661)	0.0185 (-0.0158–0.0528)	0.0185 (-0.0158–0.0528)	0.0207 (-0.0167–0.0581)
	C5	0.0196 (-0.0167–0.0559)	0.0196 (-0.0165–0.0557)	0.0196 (-0.0167–0.0559)	0.0196 (-0.0167–0.0559)	**0.6862** (0.6499–0.7225)	**0.0786** (0.0147–0.1425)	0.0196 (-0.0167–0.0559)	0.0196 (-0.0167–0.0559)	0.0196 (-0.0167–0.0559)	0.0392 (-0.0074–0.0858)	0.0196 (-0.0165–0.0557)	0.0196 (-0.0167–0.0559)	0.0196 (-0.0167–0.0559)
	C6	0.0150 (-0.0134–0.0434)	0.0151 (-0.0131–0.0433)	0.0151 (-0.0131–0.0433)	0.0152 (-0.0132–0.0436)	0.0152 (-0.0132–0.0436)	**0.8063** (0.7389–0.8737)	0.0151 (-0.0131–0.0433)	0.0151 (-0.0133–0.0435)	0.0152 (-0.0132–0.0436)	0.0270 (-0.0095–0.0635)	0.0152 (-0.0132–0.0436)	0.0152 (-0.0132–0.0436)	0.0152 (-0.0132–0.0436)
	C8	0.0152 (-0.0132–0.0436)	0.0152 (-0.0134–0.0438)	0.0152 (-0.0132–0.0436)	0.0152 (-0.0132–0.0436)	0.0151 (-0.0133–0.0435)	**0.1289** (0.0632–0.1946)	**0.6818** (0.6534–0.7102)	0.0152 (-0.0132–0.0436)	0.0152 (-0.0132–0.0436)	0.0377 (-0.0017–0.0771)	0.0152 (-0.0132–0.0436)	0.0152 (-0.0132–0.0436)	0.0151 (-0.0133–0.0435)
	C10	0.0151 (-0.0131–0.0433)	0.0151 (-0.0131–0.0433)	0.0151 (-0.0133–0.0435)	0.0152 (-0.0132–0.0436)	0.0152 (-0.0132–0.0436)	**0.1121** (0.0521–0.1721)	0.0151 (-0.0133–0.0435)	**0.6818** (0.6534–0.7102)	0.0151 (-0.0133–0.0435)	**0.0546** (0.0121–0.0971)	0.0151 (-0.0133–0.0435)	0.0151 (-0.0133–0.0435)	0.0151 (-0.0133–0.0435)
	C11	0.0175 (-0.015–0.0500)	0.0175 (-0.015–0.0500)	0.0175 (-0.015–0.0500)	0.0175 (-0.015–0.0500)	0.0175 (-0.015–0.0500)	**0.1017** (0.0358–0.1676)	0.0176 (-0.0149–0.0501)	0.0175 (-0.0152–0.0502)	**0.6842** (0.6515–0.7169)	0.0386 (-0.0049–0.0821)	0.0176 (-0.0149–0.0501)	0.0176 (-0.0149–0.0501)	0.0176 (-0.0149–0.0501)
	C12	0.0145 (-0.0127–0.0417)	0.0145 (-0.0127–0.0417)	0.0145 (-0.0127–0.0417)	0.0145 (-0.0127–0.0417)	0.0145 (-0.0127–0.0417)	**0.1258** (0.0635–0.1881)	0.0145 (-0.0127–0.0417)	0.0145 (-0.0127–0.0417)	0.0145 (-0.0127–0.0417)	**0.7145** (0.6733–0.7557)	0.0145 (-0.0127–0.0417)	0.0146 (-0.0128–0.0420)	0.0145 (-0.0127–0.0417)
	NR	0.0111 (-0.0101–0.0323)	0.0111 (-0.0101–0.0323)	0.0111 (-0.0099–0.0321)	0.0111 (-0.0101–0.0323)	0.0111 (-0.0099–0.0321)	0.0111 (-0.0101–0.0323)	0.0111 (-0.0099–0.0321)	0.0111 (-0.0099–0.0321)	0.0111 (-0.0101–0.0323)	0.0111 (-0.0099–0.0321)	**0.6778** (0.6566–0.699)	0.0111 (-0.0099–0.0321)	**0.2000** (0.1426–0.2574)
	FR	0.0152 (-0.0132–0.0436)	0.0151 (-0.0133–0.0435)	0.0152 (-0.0132–0.0436)	0.0152 (-0.0132–0.0436)	0.0152 (-0.0132–0.0436)	0.0152 (-0.0132–0.0436)	0.0152 (-0.0132–0.0436)	0.0151 (-0.0133–0.0435)	0.0152 (-0.0132–0.0436)	0.0151 (-0.0131–0.0433)	0.0152 (-0.0132–0.0436)	**0.6818** (0.6534–0.7102)	**0.1514** (0.0834–0.2194)
	G-D	0.0030 (-0.0029–0.0089)	0.0030 (-0.0029–0.0089)	0.0030 (-0.0029–0.0089)	0.0030 (-0.0029–0.0089)	0.0030 (-0.0029–0.0089)	0.0030 (-0.0029–0.0089)	0.0030 (-0.0027–0.0087)	0.0030 (-0.0029–0.0089)	0.0030 (-0.0029–0.0089)	0.0030 (-0.0029–0.0089)	0.0030 (-0.0029–0.0089)	0.0030 (-0.0029–0.0089)	**0.9639** (0.9447–0.9831)

Abbreviations as follows: NR = Neales River, FR = Frome River.

Table 21. Recent migration rates of bony herring within the Georgina-Diamantina River. Values on the diagonal are percentages of resident individuals. Other values are migration rates from sites in columns into sites in rows. 95% credible intervals in parentheses. Bold values are significantly greater than zero (95% CI excludes zero).

		Source Population																
		G1	**G2**	**G3**	**G4**	**G5**	**G6**	**G7**	**G8**	**G10**	**G11**	**G12**	**G14**	**G16a**	**G19**	**NR**	**FR**	**CC**
Sampled Population	**G1**	**0.6848** (0.6397–0.7299)	0.0326 (-0.0046–0.0698)	**0.1035** (0.0329–0.1741)	0.0119 (-0.0106–0.0344)	0.0119 (-0.0106–0.0344)	0.0119 (-0.0106–0.0344)	0.0120 (-0.0105–0.0345)	0.0119 (-0.0104–0.0342)	0.0119 (-0.0106–0.0344)	0.0119 (-0.0106–0.0344)	0.0119 (-0.0106–0.0344)	0.0119 (-0.0106–0.0344)	0.0119 (-0.0106–0.0344)	0.0119 (-0.0106–0.0344)	0.0123 (-0.0110–0.0356)	0.0119 (-0.0106–0.0344)	0.0119 (-0.0106–0.0344)
	G2	0.0119 (-0.0106–0.0344)	**0.7962** (0.7368–0.8556)	0.0133 (-0.0112–0.0378)	0.0119 (-0.0106–0.0344)	0.0120 (-0.0105–0.0345)	0.0119 (-0.0106–0.0344)	0.0119 (-0.0104–0.0342)	0.0119 (-0.0104–0.0342)	0.0119 (-0.0106–0.0344)	0.0118 (-0.0105–0.0341)	0.0120 (-0.0105–0.0345)	0.0119 (-0.0106–0.0344)	0.0119 (-0.0104–0.0342)	0.0119 (-0.0104–0.0342)	0.0119 (-0.0106–0.0344)	0.0119 (-0.0106–0.0344)	0.0119 (-0.0106–0.0344)
	G3	0.0119 (-0.0106–0.0344)	0.0119 (-0.0106–0.0344)	**0.7794** (0.7202–0.8386)	0.0119 (-0.0106–0.0344)	0.0118 (-0.0107–0.0343)	0.0119 (-0.0106–0.0344)	0.0119 (-0.0106–0.0344)	0.0119 (-0.0106–0.0344)	0.0119 (-0.0106–0.0344)	0.0119 (-0.0106–0.0344)	0.0119 (-0.0104–0.0342)	0.0120 (-0.0105–0.0345)	0.0119 (-0.0106–0.0344)	0.0119 (-0.0106–0.0344)	0.0302 (-0.0021–0.0625)	0.0119 (-0.0106–0.0344)	0.0119 (-0.0106–0.0344)
	G4	0.0119 (-0.0106–0.0344)	0.0119 (-0.0106–0.0344)	**0.1277** (0.0681–0.1873)	**0.6786** (0.6561–0.7011)	0.0119 (-0.0106–0.0344)	0.0119 (-0.0106–0.0344)	0.0119 (-0.0106–0.0344)	0.0119 (-0.0106–0.0344)	0.0119 (-0.0104–0.0342)	0.0119 (-0.0106–0.0344)	0.0119 (-0.0106–0.0344)	0.0119 (-0.0104–0.0342)	0.0119 (-0.0106–0.0344)	0.0119 (-0.0106–0.0344)	0.0151 (-0.0116–0.0418)	0.0119 (-0.0106–0.0344)	0.0119 (-0.0106–0.0344)
	G5	0.0175 (-0.0150–0.0500)	0.0175 (-0.0150–0.0500)	0.0299 (-0.0120–0.0718)	0.0176 (-0.0151–0.0503)	**0.6842** (0.6517–0.7167)	0.0176 (-0.0151–0.0503)	0.0176 (-0.0151–0.0503)	0.0176 (-0.0151–0.0503)	0.0175 (-0.0152–0.0502)	0.0176 (-0.0151–0.0503)	0.0175 (-0.0150–0.0500)	0.0175 (-0.0150–0.0500)	0.0175 (-0.0150–0.0500)	0.0176 (-0.0151–0.0503)	0.0228 (-0.0142–0.0598)	0.0175 (-0.0150–0.0500)	0.0175 (-0.0150–0.0500)
	G6	0.0119 (-0.0106–0.0344)	0.0119 (-0.0104–0.0342)	0.1219 (0.0637–0.1801)	0.0119 (-0.0106–0.0344)	0.0119 (-0.0106–0.0344)	**0.6785** (0.6560–0.7010)	0.0118 (-0.0105–0.0341)	0.0119 (-0.0106–0.0344)	0.0119 (-0.0104–0.0342)	0.0119 (-0.0106–0.0344)	0.0118 (-0.0105–0.0341)	0.0119 (-0.0106–0.0344)	0.0119 (-0.0106–0.0344)	0.0119 (-0.0106–0.0344)	0.0214 (-0.0058–0.0486)	0.0119 (-0.0106–0.0344)	0.0119 (-0.0106–0.0344)
	G7	0.0133 (-0.0118–0.0384)	0.0133 (-0.0118–0.0384)	**0.1034** (0.044–0.1628)	0.0133 (-0.0118–0.0384)	0.0133 (-0.0118–0.0384)	0.0133 (-0.0118–0.0384)	**0.6800** (0.6549–0.7051)	0.0133 (-0.0118–0.0384)	0.0134 (-0.0117–0.0385)	0.0134 (-0.0117–0.0385)	0.0133 (-0.0118–0.0384)	0.0133 (-0.0118–0.0384)	0.0133 (-0.0118–0.0384)	0.0133 (-0.0118–0.0384)	0.0167 (-0.0113–0.0447)	0.0134 (-0.0117–0.0385)	0.0133 (-0.0118–0.0384)
	G8	0.0175 (-0.0152–0.0502)	0.0175 (-0.0150–0.0500)	0.0316 (-0.0109–0.0741)	0.0176 (-0.0149–0.0501)	0.0176 (-0.0151–0.0503)	0.0175 (-0.0150–0.0500)	0.0176 (-0.0149–0.0501)	**0.6842** (0.6517–0.7167)	0.0175 (-0.0152–0.0502)	0.0175 (-0.0150–0.0500)	0.0176 (-0.0151–0.0503)	0.0176 (-0.0151–0.0503)	0.0175 (-0.0150–0.0500)	0.0176 (-0.0151–0.0503)	0.0211 (-0.0142–0.0564)	0.0176 (-0.0151–0.0503)	0.0175 (-0.0150–0.0500)
	G10	0.0175 (-0.0152–0.0502)	0.0176 (-0.0149–0.0501)	0.0301 (-0.012–0.0722)	0.0175 (-0.0150–0.0500)	0.0176 (-0.0151–0.0503)	0.0175 (-0.0150–0.0500)	0.0176 (-0.0151–0.0503)	0.0176 (-0.0151–0.0503)	**0.6842** (0.6515–0.7169)	0.0176 (-0.0151–0.0503)	0.0176 (-0.0149–0.0501)	0.0175 (-0.0152–0.0502)	0.0175 (-0.0148–0.0498)	0.0175 (-0.0150–0.0500)	0.0225 (-0.0143–0.0593)	0.0175 (-0.0152–0.0502)	0.0176 (-0.0151–0.0503)
	G11	0.0120 (-0.0105–0.0345)	0.0119 (-0.0106–0.0344)	**0.0695** (0.0248–0.1142)	0.0119 (-0.0106–0.0344)	0.0119 (-0.0106–0.0344)	0.0119 (-0.0106–0.0344)	0.0119 (-0.0106–0.0344)	0.0119 (-0.0106–0.0344)	0.0119 (-0.0106–0.0344)	**0.6786** (0.6561–0.7011)	0.0120 (-0.0105–0.0345)	0.0119 (-0.0106–0.0344)	0.0119 (-0.0106–0.0344)	0.0119 (-0.0106–0.0344)	**0.0731** (0.0290–0.1172)	0.0120 (-0.0105–0.0345)	0.0119 (-0.0106–0.0344)
	G12	0.0128 (-0.0113–0.0369)	0.0128 (-0.0115–0.0371)	**0.0657** (0.0177–0.1137)	0.0128 (-0.0113–0.0369)	0.0128 (-0.0113–0.0369)	0.0128 (-0.0113–0.0369)	0.0128 (-0.0113–0.0369)	0.0128 (-0.0113–0.0369)	0.0129 (-0.0114–0.0372)	0.0127 (-0.0114–0.0368)	**0.6795** (0.6554–0.7036)	0.0129 (-0.0114–0.0372)	0.0128 (-0.0115–0.0371)	0.0129 (-0.0112–0.0370)	**0.0627** (0.0157–0.1097)	0.0128 (-0.0113–0.0369)	0.0128 (-0.0115–0.0371)
	G14	0.0158 (-0.0138–0.0454)	0.0158 (-0.0138–0.0454)	0.0383 (-0.0064–0.083)	0.0159 (-0.0137–0.0455)	0.0159 (-0.0137–0.0455)	0.0159 (-0.0139–0.0457)	0.0160 (-0.0138–0.0458)	0.0159 (-0.0139–0.0457)	0.0158 (-0.0138–0.0454)	0.0159 (-0.0139–0.0457)	0.0159 (-0.0137–0.0455)	**0.6825** (0.6529–0.7121)	0.0158 (-0.0138–0.0454)	0.0159 (-0.0137–0.0455)	0.0409 (-0.005–0.0868)	0.0159 (-0.0139–0.0457)	0.0158 (-0.0138–0.0454)
	G16a	0.0124 (-0.0109–0.0357)	0.0124 (-0.0111–0.0359)	**0.1006** (0.0449–0.1563)	0.0124 (-0.0109–0.0357)	0.0123 (-0.011–0.0356)	0.0124 (-0.0109–0.0357)	0.0124 (-0.0109–0.0357)	0.0123 (-0.0110–0.0356)	0.0124 (-0.0109–0.0357)	0.0124 (-0.0109–0.0357)	0.0123 (-0.011–0.0356)	0.0124 (-0.0109–0.0357)	**0.6790** (0.6557–0.7023)	0.0124 (-0.0109–0.0357)	**0.0348** (0.0011–0.0685)	0.0124 (-0.0111–0.0359)	0.0124 (-0.0109–0.0357)
	G19	0.0159 (-0.0139–0.0457)	0.0158 (-0.0138–0.0454)	0.0215 (-0.0126–0.0556)	0.0159 (-0.0137–0.0455)	0.0159 (-0.0139–0.0457)	0.0159 (-0.0139–0.0457)	0.0158 (-0.0138–0.0454)	0.0158 (-0.0138–0.0454)	0.0159 (-0.0139–0.0457)	0.0159 (-0.0139–0.0457)	0.0159 (-0.0137–0.0455)	0.0159 (-0.0139–0.0457)	0.0159 (-0.0137–0.0455)	**0.6857** (0.6514–0.72)	**0.0548** (0.0021–0.1075)	0.0159 (-0.0137–0.0455)	0.0159 (-0.0137–0.0455)
	NR	0.0095 (-0.0087–0.0277)	0.0095 (-0.0087–0.0277)	**0.0760** (0.0284–0.1236)	0.0095 (-0.0085–0.0275)	0.0095 (-0.0087–0.0277)	0.0095 (-0.0087–0.0277)	0.0095 (-0.0085–0.0275)	0.0096 (-0.0086–0.0278)	0.0095 (-0.0085–0.0275)	0.0096 (-0.0086–0.0278)	0.0095 (-0.0087–0.0277)	0.0094 (-0.0086–0.0274)	0.0095 (-0.0085–0.0275)	0.0096 (-0.0086–0.0278)	**0.7715** (0.7158–0.8272)	0.0095 (-0.0085–0.0275)	0.0096 (-0.0084–0.0276)
	FR	0.0124 (-0.0109–0.0357)	0.0123 (-0.011–0.0356)	0.0263 (-0.0055–0.0581)	0.0123 (-0.011–0.0356)	0.0123 (-0.011–0.0356)	0.0123 (-0.011–0.0356)	0.0124 (-0.0109–0.0357)	0.0124 (-0.0109–0.0357)	0.0124 (-0.0109–0.0357)	0.0124 (-0.0109–0.0357)	0.0123 (-0.011–0.0356)	0.0123 (-0.011–0.0356)	0.0123 (-0.011–0.0356)	0.0123 (-0.011–0.0356)	0.1035 (0.0472–0.1598)	**0.6790** (0.6557–0.7023)	0.0183 (-0.0095–0.0461)
	CC	0.0038 (-0.0035–0.0111)	0.0038 (-0.0035–0.0111)	0.0038 (-0.0036–0.0112)	0.0038 (-0.0035–0.0111)	0.0038 (-0.0036–0.0112)	0.0038 (-0.0035–0.0111)	0.0038 (-0.0036–0.0112)	0.0039 (-0.0035–0.0113)	0.0038 (-0.0035–0.0111)	0.0038 (-0.0035–0.0111)	0.0037 (-0.0036–0.0110)	0.0038 (-0.0035–0.0111)	0.0038 (-0.0035–0.0111)	0.0038 (-0.0035–0.0111)	0.0045 (-0.0033–0.0123)	0.0038 (-0.0035–0.0111)	**0.9348** (0.9072–0.9624)

Abbreviations as follows: NR = Neales River, FR = Frome River, CC = Cooper Creek.

Appendix Three

Supporting Information for Chapter Six

Appendix 3.1 – Sampling Information

Table 1. Sampling sites in each of the seven study river systems of the Lake Eyre Basin, including geographic coordinates and number of samples of each of five study taxa (G: Finke goby *Chlamydogobius japalpa* & desert goby *Chlamydogobius eremius*; H: Finke hardyhead *Craterocephalus centralis* & Lake Eyre hardyhead *Craterocephalus eyresii*; BG: barred grunter *Amniataba percoides*; SP: spangled perch *Leiopotherapon unicolor*; BH: bony herring *Nematalosa erebi*) included in this study. Site codes are mapped in Fig. 1.

Sampling Site:	Latitude:	Longitude:	G	H	BG	SP	BH
Finke River							
1 Ellery Creek Big Hole	23°46'39"S	133°04'24"E	-	-	10	5	-
2 Bowmans Gap	23°36'51"S	132°45'33"E	-	-	-	7	8
2 Ormiston Gorge	23°37'44"S	132°43'39"E	-	-	3	3	8
2 Pioneer Creek Mound Spring	23°40'53"S	132°43'21"E	9	-	-	-	-
2 Two Mile Upper	23°40'09"S	132°40'11"E	13	11	8	10	-
2 Glen Helen Gorge	23°41'13"S	132°40'25"E	10	14	7	10	-
3 Boggy Hole	24°08'25"S	132°52'08"E	10	20	3	11	-
4 Running Waters	24°18'29"S	132°54'10"E	3	10	9	1	5
5 Three Mile Waterhole	24°30'50"S	133°13'18"E	10	5	10	-	10
5 Snake Hole	24°33'32"S	133°18'39"E	10	10	-	5	3
6 Idracowra Station	25°00'09"S	133°47'32"E	1	-	2	-	-
7 Lilla Creek Station	25°27'05"S	134°13'30"E	-	-	-	2	1
Macumba River							
8 Eringa Waterhole	26°17'15"S	134°43'45"E	-	-	-	5	3
9 Macumba Homestead	27°12'29"S	135°41'52"E	-	-	-	-	5
10 Andaranna Waterhole	27°39'10"S	136°44'30"E	-	-	2	-	-
Georgina-Diamantina							
11 Cowarie Crossing	27°36'50"S	138°18'19"E	16	-	4	6	9
12 North Ultoomurra	27°09'14"S	138°43'35"E	8	5	1	-	-
13 Brumbie Waterhole	25°39'17"S	139°50'12"E	-	-	-	-	8
14 Mulligan River	23°55'54"S	138°38'24"E	-	5	-	-	-
15 King Creek near Bedourie	24°31'58"S	139°33'53"E	-	-	1	10	7
16 Police Barracks	22°43'11"S	140°01'54"E	-	-	5	10	10
17 Tobermoray	22°16'26"S	137°58'36"E	-	-	-	-	10
18 Lake Nash	20°58'04"S	137°55'53"E	-	-	-	-	10

Cooper Creek							
19 Cuttapirra Waterhole	28°33'00"S	138°04'52"E	-	-	-	-	10
20 Lake Hope Camp	28°22'48"S	139°14'57"E	-	1	-	-	6
21 Tirra Warra Waterhole	27°26'00"S	140°08'58"E	-	-	-	-	9
22 Durham Downs	27°03'08"S	141°54'13"E	-	-	-	-	9
23 Windorah Bridge	25°22'12"S	142°44'34"E	-	-	-	10	9
24 Killman Waterhole	24°16'36"S	144°22'09"E	-	-	-	2	4
25 Stonehenge	24°21'02"S	143°15'22"E	-	-	-	5	5
26 Agricultural College	23°21'04"S	144°19'45"E	-	-	-	5	4
27 Lammermoor	21°20'35"S	144°38'51"E	-	-	-	5	9
Frome River							
28 Birdsville Track Crossing	29°38'47"S	138°04'16"E	9	3	-	1	9
South-West Creeks							
29 Finniss Creek Crossing	29°36'36"S	137°27'29"E	10	10	-	-	-
30 Screechowl Creek Crossing	29°37'39"S	137°20'09"E	9	7	-	-	-
31 Margaret Creek Crossing	29°29'24"S	137°02'21"E	6	10	-	1	-
32 Warriner Creek Crossing	29°08'16"S	136°34'06"E	7	7	-	-	-
Neales River							
33 Levi Creek	28°19'03"S	136°16'15"E	10	-	-	-	-
34 Tardetakarinna Waterhole	28°00'55"S	136°08'16"E	6	-	-	-	-
35 North Freeling Spring	28°03'04"S	135°53'32"E	6	-	-	10	-
35 Warrarawoona Waterhole	28°02'34"S	135°54'12"E	-	-	4	-	8
36 Peake Creek Rail Bridge	28°02'07"S	135°47'59"E	5	8	9	8	2
37 Algebuckina Waterhole	27°54'00"S	135°48'52"E	5	10	5	1	7
37 Ockenden Spring	27°50'32"S	135°44'31"E	6	-	-	-	-
38 Stewart Waterhole	27°41'10"S	135°22'57"E	5	6	-	1	-

Appendix 3.2 – Nucleotide Diversity Statistics

Patterns of nucleotide diversity across loci for each fish taxa.

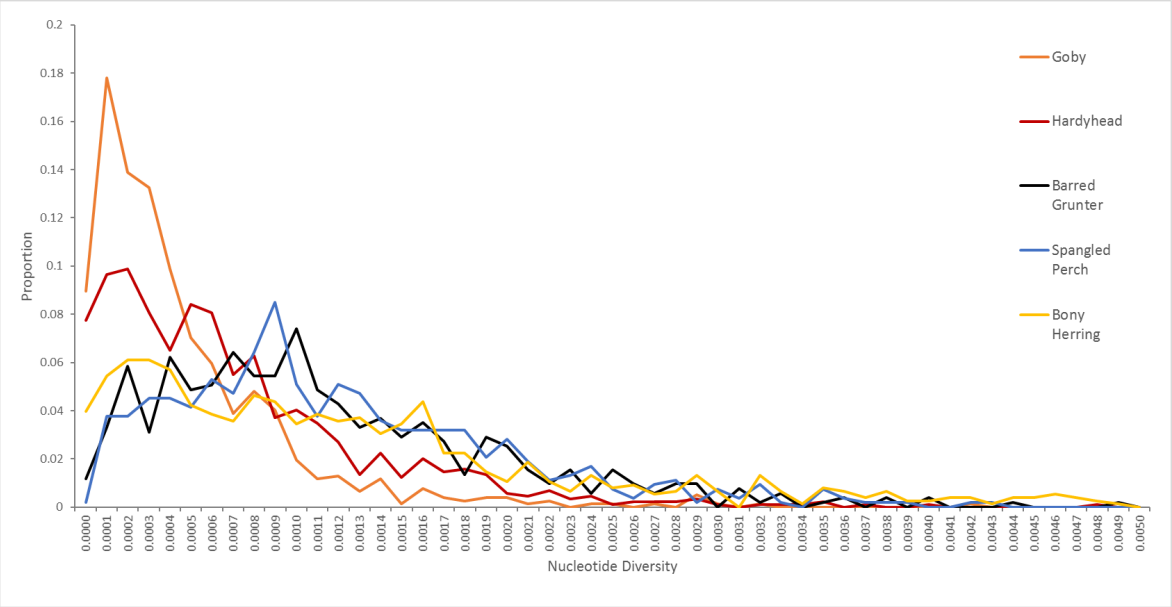

Figure 1. Nucleotide diversity of nuclear loci sampled in five fish taxa using the MetaPrep approach. Note that barred grunter and spangled perch have fewer non-diverse loci, and that the peak values for these species is an order of magnitude greater, than the other three taxa.

Appendix 3.3 – Population Structure Statistics and Visualisations

Population structure results at varying Ks and pairwise population differentiation values for each fish taxa.

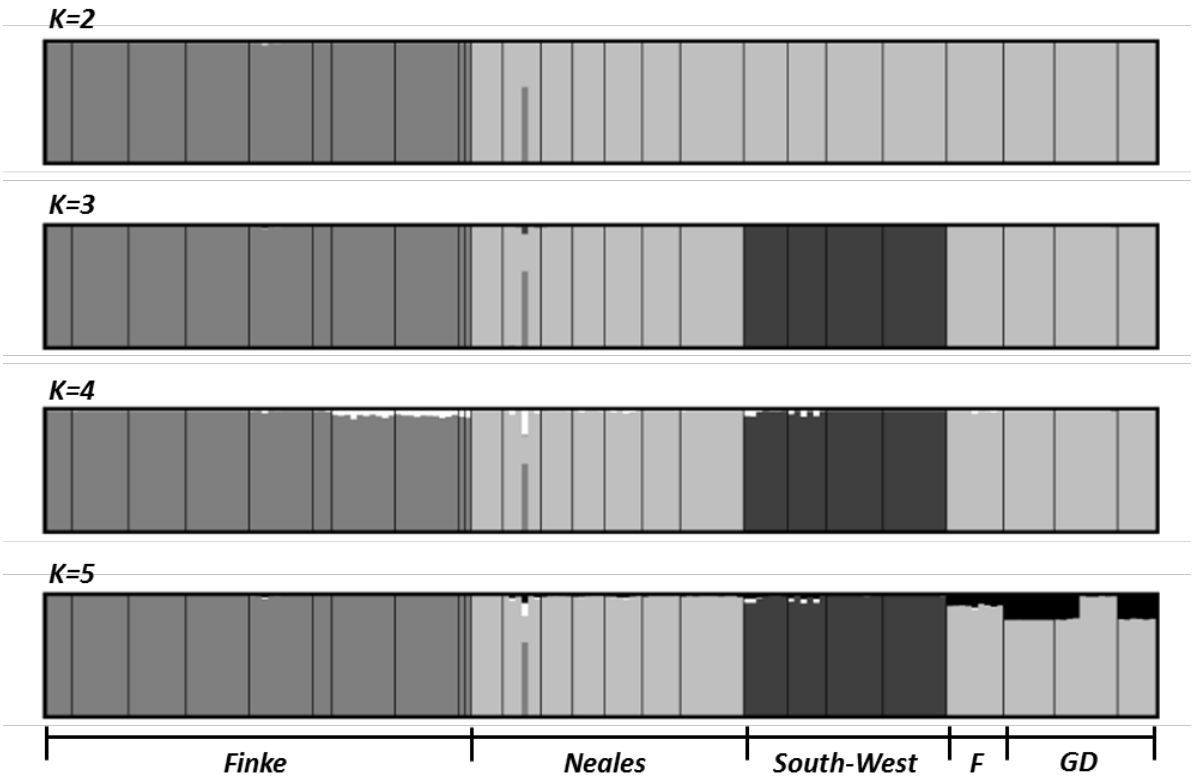

Figure 1. Population structure of desert goby *Chlamydogobius eremius* and Finke goby *Ch. japalpa* in the Lake Eyre Basin for two to five genetic clusters (the most likely K=3, see main results section), visualised with CLUMPAK, from analysis with the Bayesian individual-based non-spatial clustering algorithm STRUCTURE. Genetic clusters are represented by distinct colours, and each vertical bar shows the proportion of an individual's genotype assigned to each cluster. Sampling sites (separated by thin lines) within the following river systems (indicated on the *x*-axis) are included: Finke River, Neales River, South-West Creeks, Frome River (F), and Georgina-Diamantina River (GD).

Table 1. Nei's pairwise F_{ST} values and *p*-values (italicised, * <0.05, ** <0.01) for each river-scale population of desert and Finke goby, *Chlamydogobius eremius* and *C. japalpa*, sampled in the Lake Eyre Basin.

	Finke	**Neales**	**South-west**	**Frome**	**Geo-Dia**
Finke	-	*0.0010*	*0.0010*	*0.0010*	*0.0010*
Neales	0.6189**	-	*0.0010*	*0.0939*	*0.0070*
South-west	0.6036**	0.3331**	-	*0.0010*	*0.0010*
Frome	0.7342**	0.0593	0.3136**	-	*0.0100*
Geo-Dia	0.7953**	0.1716**	0.4264**	0.1631*	-

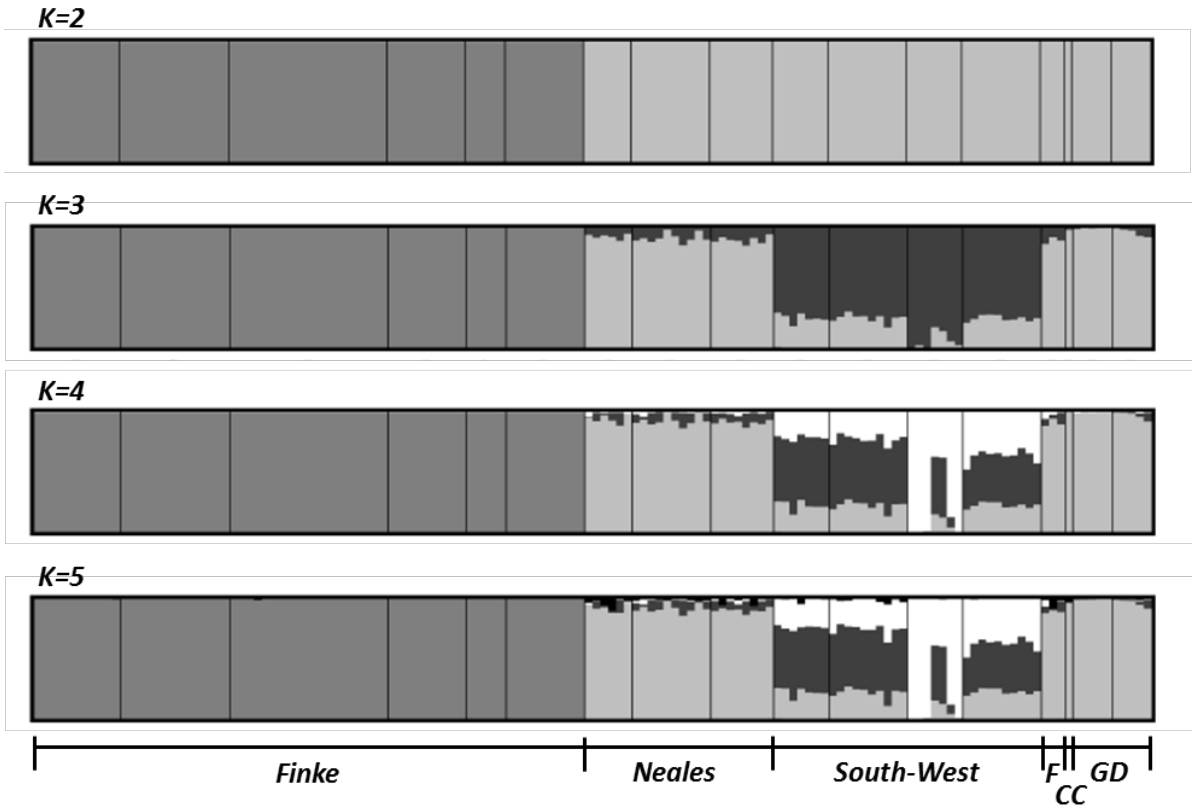

Figure 2. Population structure of Lake Eyre hardyhead *Craterocephalus eyresii* and Finke hardyhead *Cr. centralis* in the Lake Eyre Basin for two to five genetic clusters (the most likely K=2, see main results section), visualised with CLUMPAK, from analysis with the Bayesian individual-based non-spatial clustering algorithm STRUCTURE. Genetic clusters are represented by distinct colours, and each vertical bar shows the proportion of an individual's genotype assigned to each cluster. Sampling sites (separated by thin lines) within the following river systems (indicated on the *x*-axis) are included: Finke River, Neales River, South-West Creeks, Frome River (F), Cooper Creek (CC), and Georgina-Diamantina River (GD).

Table 2. Nei's pairwise F_{ST} values and *p*-values (italicised, * <0.05, ** <0.01) for each river-scale population of Lake Eyre and Finke hardyhead, *Craterocephalus eyresii* and *C. centralis*, sampled in the Lake Eyre Basin.

	Finke	**Neales**	**South-west**	**Frome**	**Cooper**	**Geo-Dia**
Finke	-	*0.0010*	*0.0120*	*0.0250*	*0.1169*	*0.0230*
Neales	0.2291**	-	*0.0519*	*0.3726*	*0.4855*	*0.5624*
South-west	0.1232*	0.0856	-	*0.0230*	*0.0430*	*0.3207*
Frome	0.1212*	0.0463	0.1708*	-	*0.2507*	*0.7053*
Cooper	0.0776	0.0446	0.1903*	0.1363	-	*0.8641*
Geo-Dia	0.2392*	0.0460	0.1144	0.0667	0.0616	-

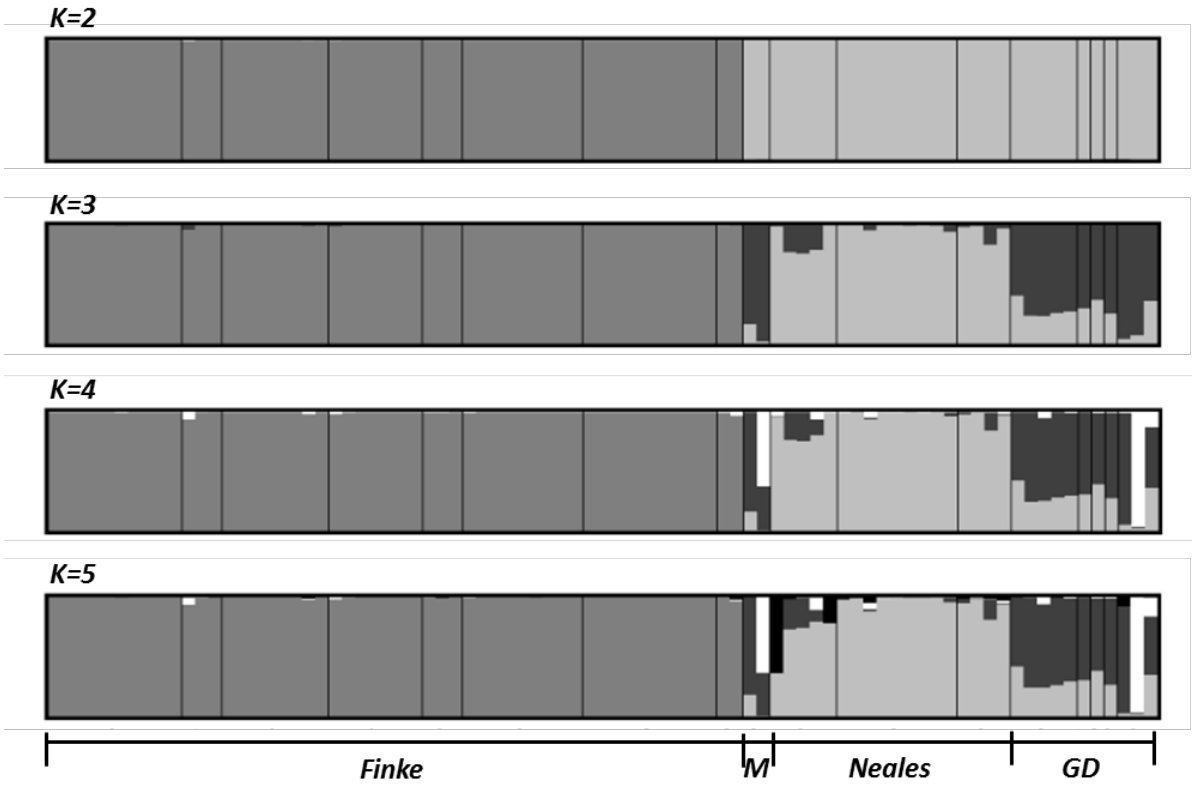

Figure 3. Population structure of barred grunter *Amniataba percoides* in the Lake Eyre Basin for two to five genetic clusters (the most likely K=2, see main results section), visualised with CLUMPAK, from analysis with the Bayesian individual-based non-spatial clustering algorithm STRUCTURE. Genetic clusters are represented by distinct colours, and each vertical bar shows the proportion of an individual's genotype assigned to each cluster. Sampling sites (separated by thin lines) within the following river systems (indicated on the *x*-axis) are included: Finke River, Macumba River (M), Neales River, and Georgina-Diamantina River (GD).

Table 3. Nei's pairwise F_{ST} values and *p*-values (italicised, * <0.05, ** <0.01) for each river-scale population of barred grunter, *Amniataba percoides*, sampled in the Lake Eyre Basin.

	Finke	**Macumba**	**Neales**	**Geo-Dia**
Finke	-	*0.0500*	*0.0010*	*0.0010*
Macumba	0.0512*	-	*0.1698*	*0.1189*
Neales	0.2082**	0.0411	-	*0.0899*
Geo-Dia	0.1693**	0.0481	0.0567	-

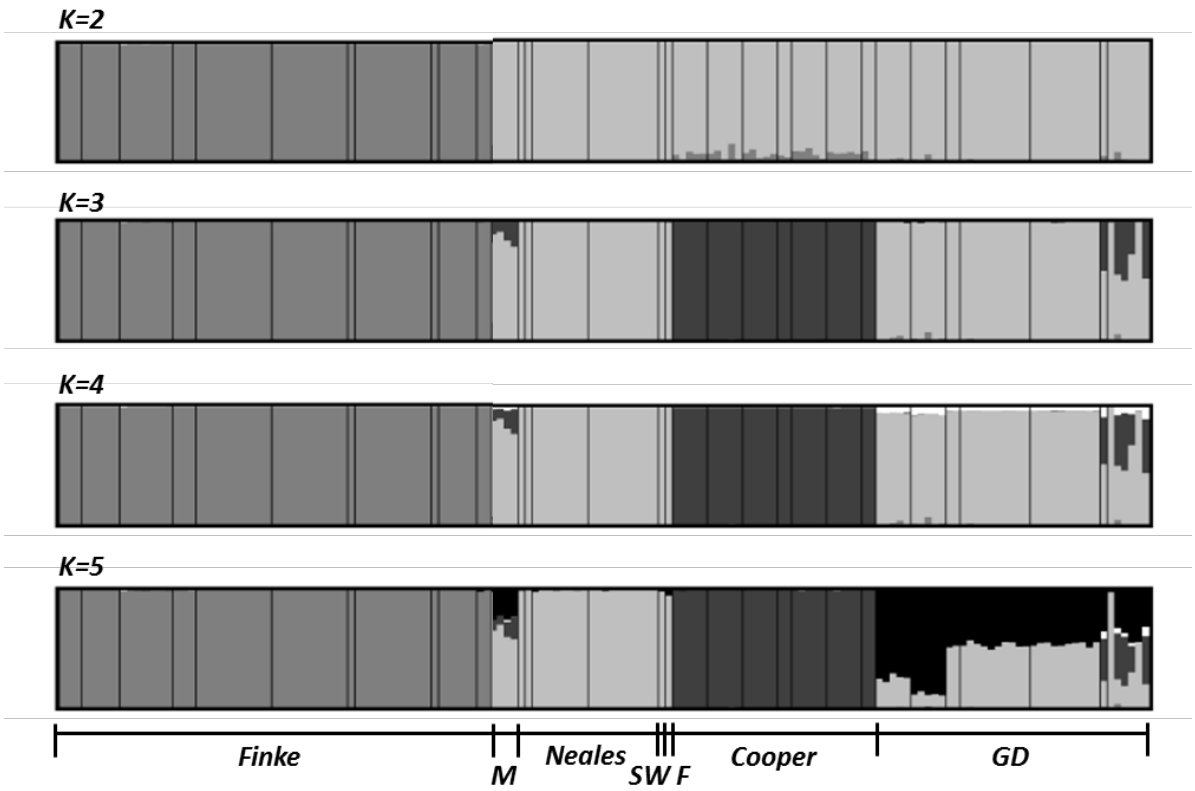

Figure 4. Population structure of spangled perch *Leiopotherapon unicolor* in the Lake Eyre Basin for two to five genetic clusters (the most likely K=2, see main results section), visualised with CLUMPAK, from analysis with the Bayesian individual-based non-spatial clustering algorithm STRUCTURE. Genetic clusters are represented by distinct colours, and each vertical bar shows the proportion of an individual's genotype assigned to each cluster. Sampling sites (separated by thin lines) within the following river systems (indicated on the *x*-axis) are included: Finke River, Macumba River (M), Neales River, South-West Creeks (SW), Frome River (F), Cooper Creek, and Georgina-Diamantina River (GD).

Table 4. Nei's pairwise F_{ST} values and *p*-values (italicised, * <0.05, ** <0.01) for each river-scale population of spangled perch, *Leiopotherapon unicolor*, sampled in the Lake Eyre Basin.

	Finke	**Macumba**	**Neales**	**South-w**	**Frome**	**Cooper**	**Geo-Dia**
Finke	-	*0.0099*	*0.0010*	*0.9800*	*0.9960*	*0.0040*	*0.0110*
Macumba	0.1084**	-	*0.0669*	*0.9970*	*1.0000*	*0.0200*	*0.3257*
Neales	0.2047**	0.0799	-	*1.0000*	*1.0000*	*0.0040*	*0.2308*
South-W	0.0059	-0.0510	-0.0194	-	*1.0000*	*0.9171*	*0.9301*
Frome	-0.0027	-0.0898	-0.0286	-1.2228	-	*0.9640*	*0.9191*
Cooper	0.1577**	0.1691*	0.1779**	-0.0023	-0.0178	-	*0.4196*
Geo-Dia	0.1952*	0.0551	0.0628	-0.0068	-0.0127	0.1135	-

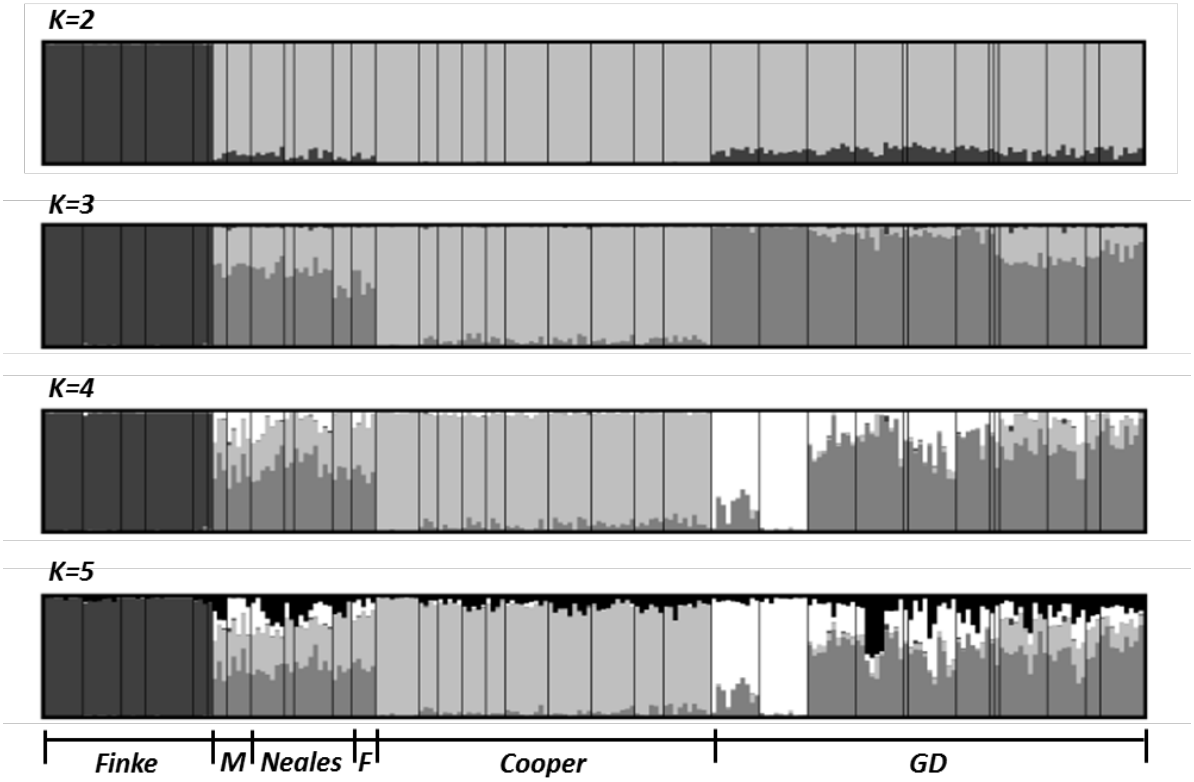

Figure 5. Population structure of bony herring *Nematalosa erebi* in the Lake Eyre Basin for two to five genetic clusters (the most likely K=2, see main results section), visualised with CLUMPAK, from analysis with the Bayesian individual-based non-spatial clustering algorithm STRUCTURE. Genetic clusters are represented by distinct colours, and each vertical bar shows the proportion of an individual's genotype assigned to each cluster. Sampling sites (separated by thin lines) within the following river systems (indicated on the *x*-axis) are included: Finke River, Macumba River (M), Neales River, Frome River (F), Cooper Creek, and Georgina-Diamantina River (GD).

Table 5. Nei's pairwise F_{ST} values and *p*-values (italicised, * <0.05, ** <0.01) for each river-scale population of bony herring, *Nematalosa erebi*, sampled in the Lake Eyre Basin.

	Finke	**Macumba**	**Neales**	**Frome**	**Cooper**	**Geo-Dia**
Finke	-	*0.0020*	*0.0010*	*0.0080*	*0.0010*	*0.0180*
Macumba	0.1191**	-	*0.0759*	*0.0120*	*0.0080*	*0.0669*
Neales	0.1137**	0.0506	-	*0.1938*	*0.0220*	*0.3836*
Frome	0.0967**	0.0789*	0.0459	-	*0.0969*	*0.0929*
Cooper	0.1626**	0.0968**	0.0786*	0.0659	-	*0.0360*
Geo-Dia	0.0960*	0.0785	0.0467	0.0801	0.0972*	-

Appendix 3.4 – Demographic History Results and IMa2p Run Information

Parameter estimates and posterior distributions of IMa2p analyses of historical demography of each fish taxa.

Table 1. Parameter estimates (mean (peak) and 95% confidence intervals) of the two-population isolation-with-migration model for five fish taxa in the Finke River and wider Lake Eyre Basin (LEB). Parameters include divergence time (t_0, years ago), population immigration rates per year ($2N_em$) forward in time into the Finke and LEB populations (m_{FINKE}, m_{LEB}), and effective population sizes of the ancestral (N_{eANC}) and contemporary (N_{eFINKE}, N_{eLEB}) populations. Asterisks indicate migration rate estimates that differ significantly from zero by LLR tests at the $P < 0.05$ level.

Taxon	t_0	m_{FINKE}	m_{LEB}	Ne_{FINKE}	Ne_{LEB}	Ne_{ANC}
Goby						
Peak	6591	0.013*	0.008	354	2480	12402
95% CI	*3614-14670*	*0.005-0.023*	*0.001-0.032*	*354-1063*	*1772-5315*	*7441-26575*
Hardyhead						
Peak	31947	0.077*	0.181*	12710	47061	50496
95% CI	*20817-50084*	*0.034-0.140*	*0.050-0.405*	*8588-19580*	*31947-69733*	*34695-76603*
Grunter						
Peak	6633	0.101*	0.960*	1622	6505	14523
95% CI	*4592-12901*	*0.045-0.190*	*0.513-1.357*	*1221-2205*	*5157-8182*	*11863-17475*
Perch						
Peak	8375	0.177*	0.719*	2940	13768	24766
95% CI	*6006-18696*	*0.084-0.316*	*0.141-1.272*	*2263-3955*	*12527-16772*	*21805-27642*
Herring						
Peak	20722	0.413*	13.050*	1250	42695	89856
95% CI	*10718-49305*	*0.253-0.491*	*6.156-37.380*	*893-5895*	*30548-71992*	*64489-121297*

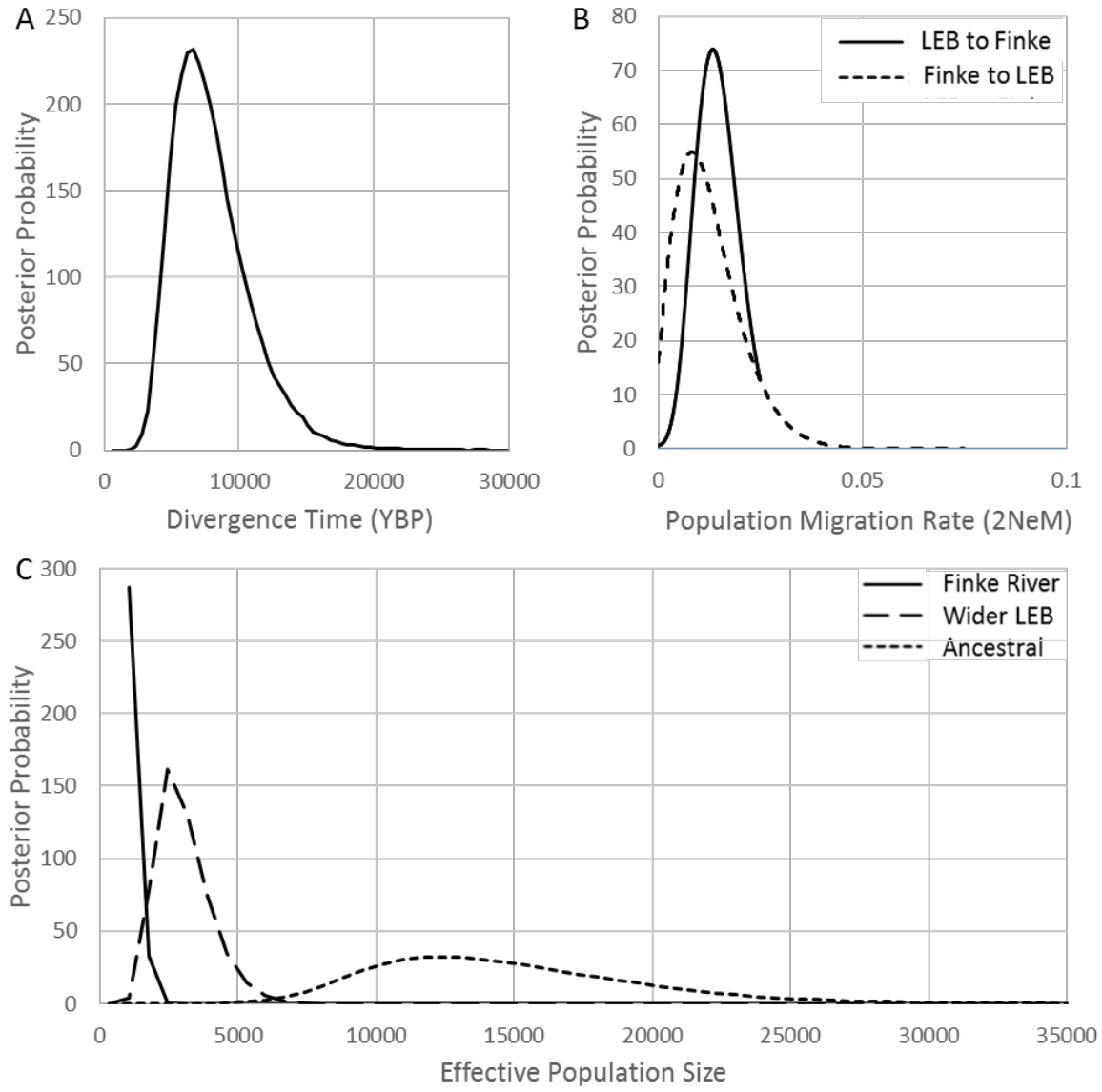

Figure 1. Posterior distributions of two-population demographic model of Finke goby, *Chlamydogobius japalpa*, and desert goby, *C. eremius*, calculated with IMa2p for 200 nuclear loci. Parameters include A) divergence times in years before present; B) population migration rates per year ($2N_em$) forward in time into the Finke River and wider Lake Eyre Basin populations; and C) effective population sizes of the contemporary (Finke River and wider Lake Eyre Basin) and ancestral populations.

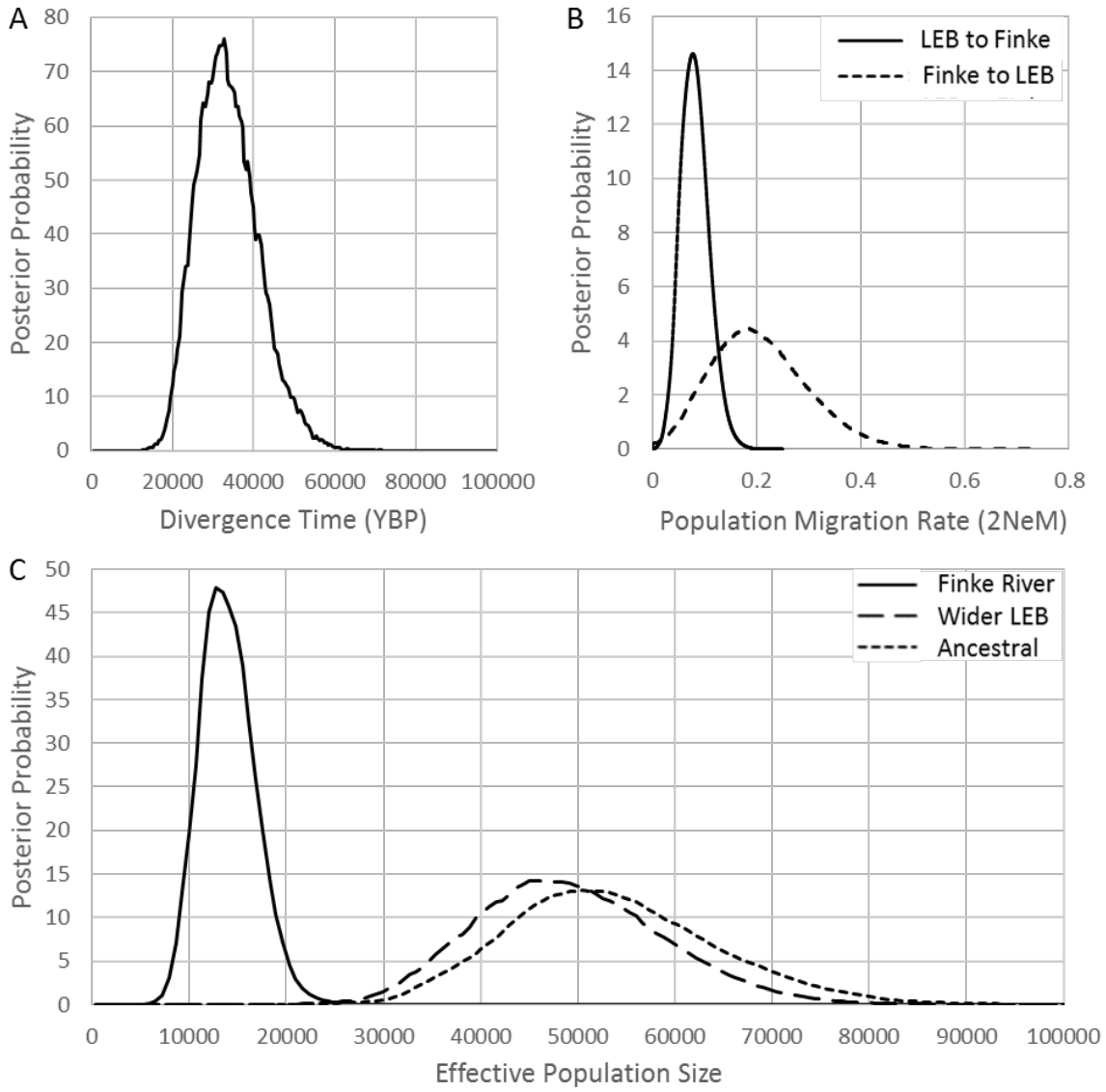

Figure 2. Posterior distributions of two-population demographic model of Finke hardyhead, *Craterocephalus centralis*, and Lake Eyre hardyhead, *C. eyresii*, calculated with IMa2p for 200 nuclear loci. Parameters include A) divergence times in years before present; B) population migration rates per year ($2N_em$) forward in time into the Finke River and wider Lake Eyre Basin populations; and C) effective population sizes of the contemporary (Finke River and wider Lake Eyre Basin) and ancestral populations.

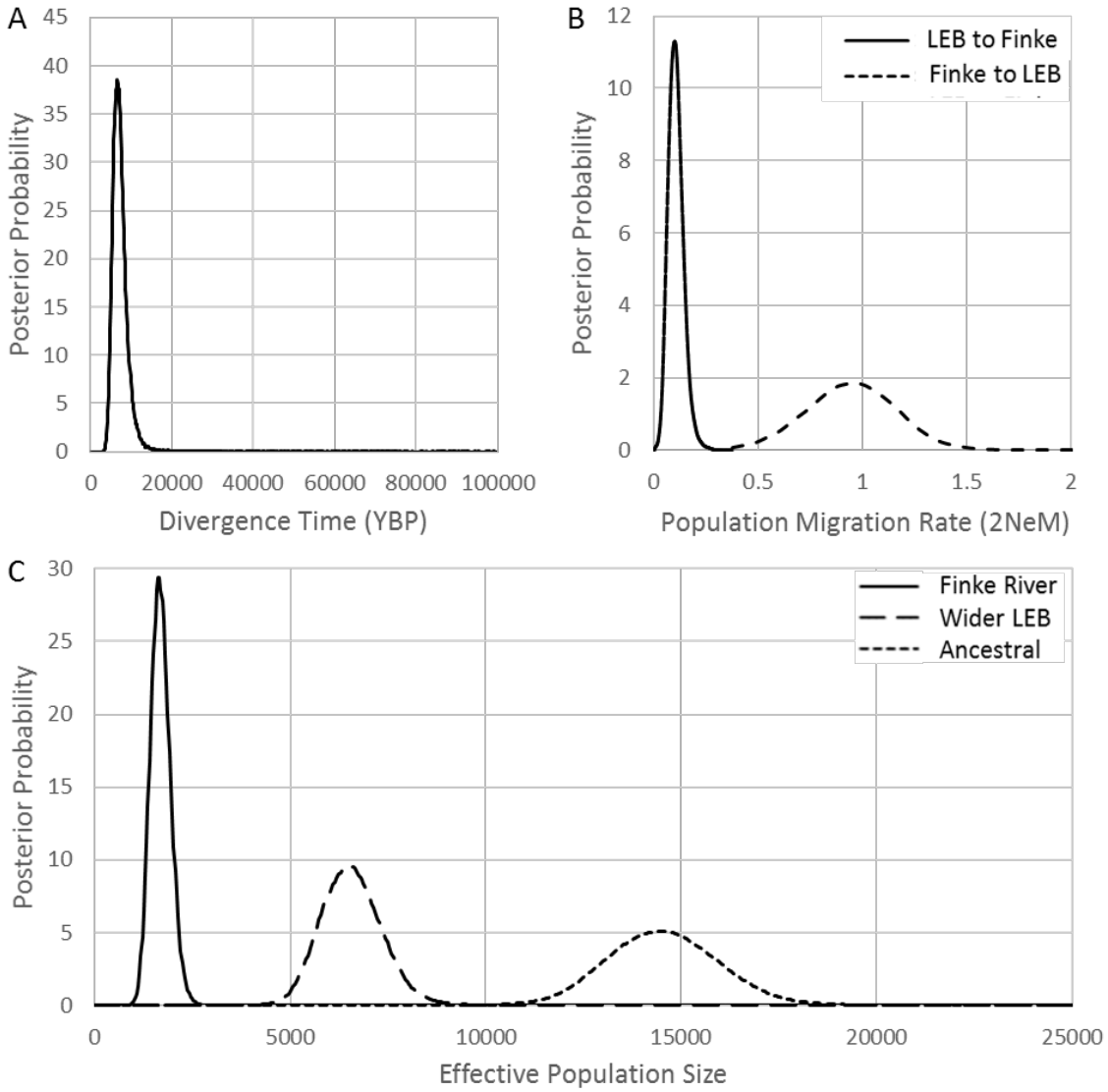

Figure 3. Posterior distributions of two-population demographic model of barred grunter, *Amniataba percoides*, calculated with IMa2p for 200 nuclear loci. Parameters include A) divergence times in years before present; B) population migration rates per year ($2N_em$) forward in time into the Finke River and wider Lake Eyre Basin populations; and C) effective population sizes of the contemporary (Finke River and wider Lake Eyre Basin) and ancestral populations.

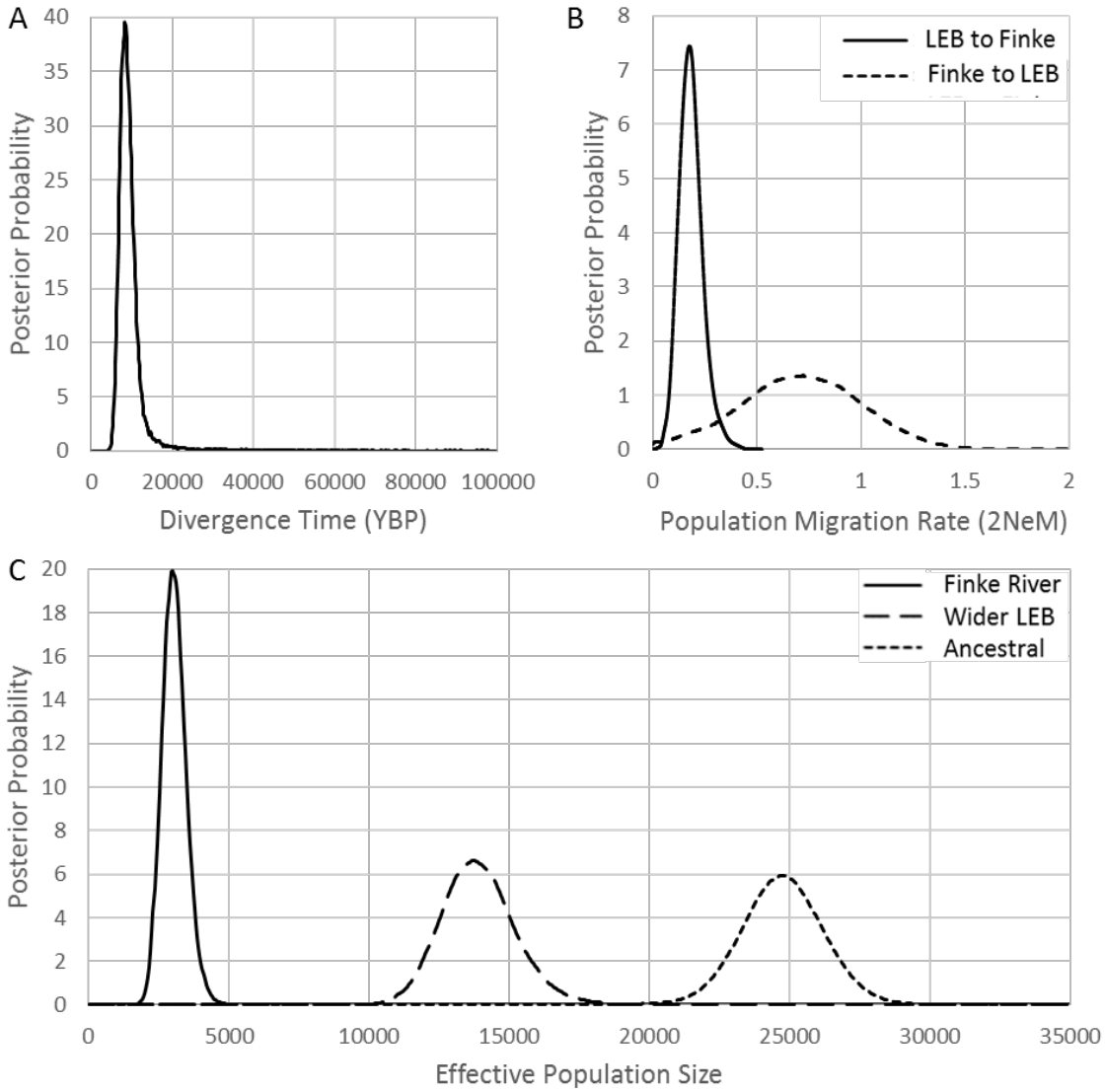

Figure 4. Posterior distributions of two-population demographic model of spangled perch, *Leiopotherapon unicolor*, calculated with IMa2p for 200 nuclear loci. Parameters include A) divergence times in years before present; B) population migration rates per year ($2N_em$) forward in time into the Finke River and wider Lake Eyre Basin populations; and C) effective population sizes of the contemporary (Finke River and wider Lake Eyre Basin) and ancestral populations.

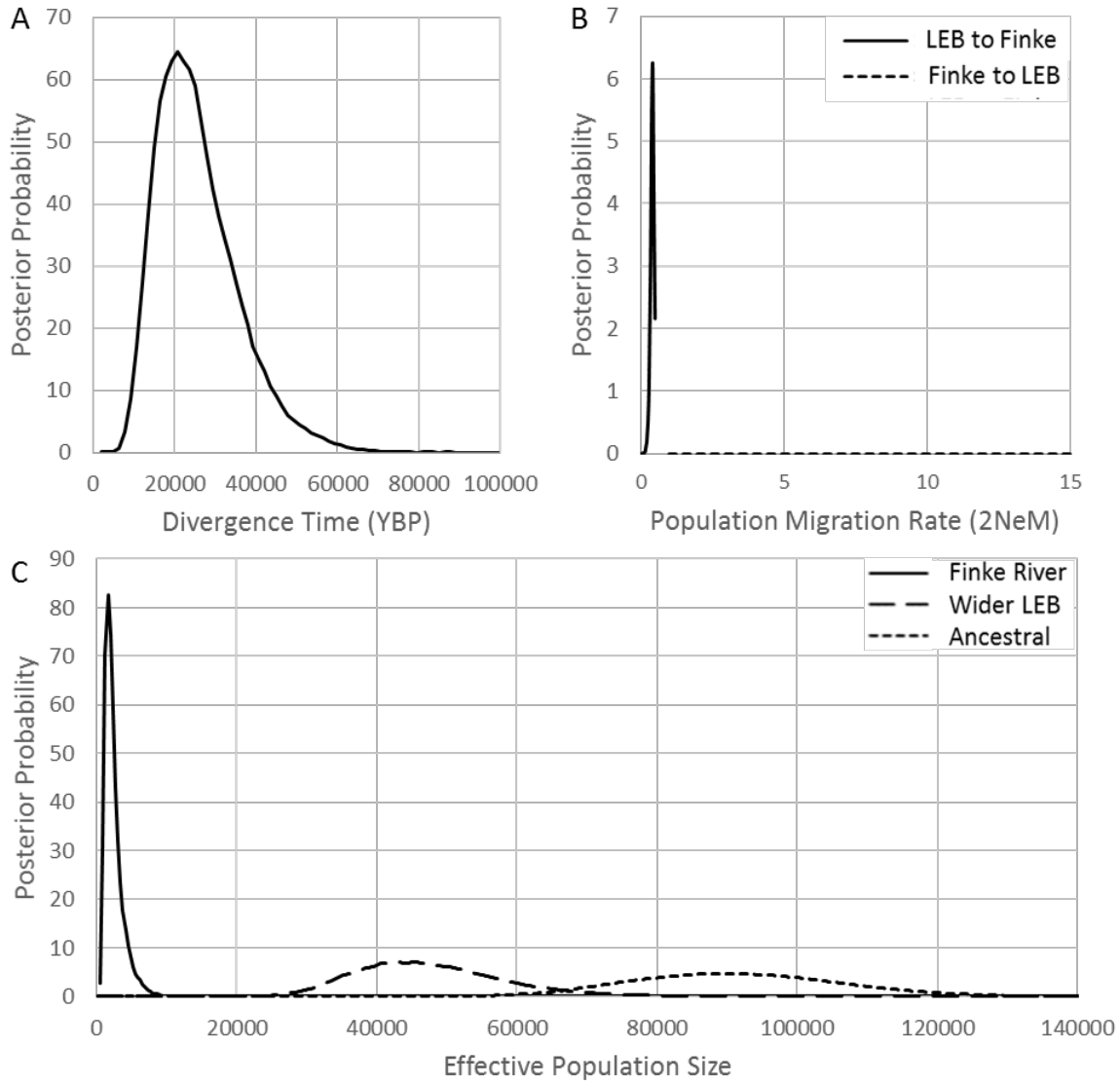

Figure 5. Posterior distributions of two-population demographic model of bony herring, *Nematalosa erebi*, calculated with IMa2p for 200 nuclear loci. Parameters include A) divergence times in years before present; B) population migration rates per year ($2N_em$) forward in time into the Finke River and wider Lake Eyre Basin populations; and C) effective population sizes of the contemporary (Finke River and wider Lake Eyre Basin) and ancestral populations.

Appendix 3.5 – Genetic Diversity Statistics and Significance Tests

Means, standard deviations and results of significance testing for genetic diversity values within the Finke River and the wider Lake Eyre Basin (LEB) for each of five taxa.

Table 1. Mean (μ) and standard deviations (SD) of genetic diversity measures for five fish taxa sampled in the Finke River and the wider LEB used to calculate effect sizes, including AR: mean allelic richness (adjusted for sample size of 5); H_O: mean observed heterozygosity; H_E: mean expected heterozygosity; π: mean nucleotide diversity (x 10^{-4}); per waterhole and then averaged for the Finke and wider LEB respectively. Only waterholes with more than five individuals scored for genetic variation included.

		AR		**H_E**		**H_O**		**π**	
Taxon	**River**	**μ**	**SD**	**μ**	**SD**	**μ**	**SD**	**μ**	**SD**
Goby	Finke	1.065	0.208	0.024	0.080	0.026	0.094	0.060	0.203
	LEB	1.586	0.410	0.216	0.140	0.209	0.146	0.516	0.547
Hardyhead	Finke	1.555	0.566	0.208	0.207	0.200	0.209	0.386	0.629
	LEB	1.944	0.608	0.318	0.183	0.327	0.197	0.661	0.714
Grunter	Finke	1.641	0.621	0.231	0.215	0.231	0.222	0.573	0.783
	LEB	2.107	0.622	0.403	0.185	0.387	0.213	1.147	1.012
Perch	Finke	1.741	0.602	0.280	0.212	0.276	0.220	0.874	0.893
	LEB	2.194	0.580	0.392	0.157	0.405	0.170	1.160	0.826
Herring	Finke	1.651	0.609	0.241	0.215	0.245	0.228	0.872	1.362
	LEB	2.105	0.584	0.349	0.168	0.360	0.179	1.282	1.323

Table 2. Results of mixed effects models to determine whether the Finke and the wider LEB differ in four genetic diversity measures, including AR: mean allelic richness (adjusted for sample size of 5); H_O: mean observed heterozygosity; H_E: mean expected heterozygosity; π: mean nucleotide diversity; per waterhole. Only waterholes with more than five individuals scored for genetic variation included. For all tests, d.f. = 1.

	AR		**H_O**		**H_E**		**π**	
Taxon	***χ^2***	***P***	***χ^2***	***P***	***χ^2***	***P***	***χ^2***	***P***
Goby	14.68	< 0.001	30.02	< 0.001	34.77	< 0.001	23.66	< 0.001
Hardyhead	27.11	< 0.001	63.18	< 0.001	42.12	< 0.001	94.74	< 0.001
Grunter	12.70	< 0.001	37.46	< 0.001	136.00	< 0.001	226.98	< 0.001
Perch	21.41	< 0.001	112.21	< 0.001	130.69	< 0.001	102.56	< 0.001
Herring	10.53	0.0012	11.33	< 0.001	10.36	0.0013	9.14	0.0025