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Making every drop count: functional connectivity and habitat use for small-bodied fish in semi-arid aquatic ecosystems

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Section 1. Literature review

1.1 Ecosystem functions, services and processes

From a local to a global scale, humans are changing the biological composition of ecosystems through a variety of activities that increase the rate of species invasions and extinctions, landscape modification and habitat fragmentation (Johnson *et al*., 2004; Hooper *et al.,* 2005; Fisher and Lindenmayer, 2007). Current ecological knowledge suggests that ecosystem properties are dependent on biodiversity and, in particular, dependent on the functional characteristics of the organisms present, how they are distributed and their abundances (Hooper *et al.,* 2005). Humans can cause alterations to all of these factors (Hooper *et al.,* 2005); here I will focus on the functional aspect of ecosystem properties.

According to Ricklefs (1984) there are five main ecological properties of a functional ecosystem: 1) biogeochemical cycles; 2) primary and secondary production; 3) mineralization of organic matter; 4) storage and transport of minerals and biomass; and 5) the regulation and continuation of these four processes. Research on ecosystem functions has become a prominent topic in ecology however, the word function is used to mean several things (Jax, 2005) and is often not well distinguished from ecosystem process. Here, I define an ecosystem process as the interaction between two objects where a state changes through time. For example, carbon entering the ecosystem through photosynthesis and being incorporated into an organism is an ecosystem process (Chapin *et al*., 2002). In contrast, an ecosystem function is the culmination of a single or several physical, chemical and biological ecosystem processes that explain how an organism interacts with other organisms, with their environment and contribute to one or all of the main ecological properties (Jax, 2005; Lester *et al*., 2011). For example, the carbon cycle where carbon is exchanged between organisms and the environment contributing to the biogeochemical cycles of an ecosystem. Another term that is often conflated with ecosystem functions is ecosystem service (Jax, 2005). Here, an ecosystem service is defined as an attribute of an ecosystem that benefits humans (Costanza, 1997). For example, the carbon though primary and secondary production creating a commercially viable fishery.

A functional approach to ecology, investigating the relationships between processes and organisms (i.e. ecosystem functions) rather than just organism assemblages or abiotic factors (i.e. ecosystem processes), has long been considered the future of conservation (Goldstein, 1999). It has even been suggested that species-specific information may not be required to understand and manage ecosystem functions (Goldstein, 1999). Relative abundance is not necessarily a good indicator of the importance of a species to ecosystem function (e.g. in the case of a rare but keystone predator) and therefore, species can strongly influence ecosystems functions (Hooper *et al.,* 2005). Thus, when considering ecological research a functional approach to ecological questions is important to gain a more holistic understanding of ecosystems.

1.2 Functional connectivity

Functional connectivity is often listed as a critical ecosystem function that influences biotic response to other biota and the landscape. Functional connectivity is the movement of individuals, as well as groups of animals or populations, through and within the landscape across space and time (Watts and Handley, 2010). Functional connectivity can act as a key determinant of the genetic composition of populations (e.g. Lambeets *et al*., 2009) and community structure (e.g. Gallardo *et al*., 2008) through movement of individuals (both emigration and immigration) among habitats. It is also a key factor for determining the viability of populations under habitat fragmentation (Pe'er *et* al., 2011).

Species richness and assemblage structure have often shown strong and positive correlations with habitat connectivity (Miyazono *et al*., 2010). Connections between habitats are dependent on individual species' habitat preferences, their requirement for specific habitat types and their accessibility to the habitat (With and Crist, 1995; Dancose *et al*., 2011). The interaction between habitat selection, preference and accessibility to each habitat gives a general understanding of functional connectivity for a species or group of similar species but has largely been poorly studied and understood (Mumby and Hastings, 2008; Dancose *et al*., 2011). Therefore, functional connectivity depends upon both a species' perception of available habitat and its use of it, as well as movements between and among habitats (Kadoya 2009; Turgeon *et al*., 2010). Therefore, functional connectivity is scale dependent, based on the size and dispersal ability of a given species (Johnson *et al*., 2004; Dancose *et al*., 2011; Cushman and Landguth, 2012). Furthermore, landscape elements that inhibit movement (e.g. dams; With and Crist, 1995; Cushman and Landguth, 2012) and the density and distribution of the populations among the habitats can affect connectivity (Cushman and Landguth, 2012). As the areas of habitat become smaller, connectivity among habitats may become disrupted and negative impacts on the distribution and persistence of populations typically occur (With and Crist, 1995).

It is presumed that habitat specialists with limited dispersal ability will be more greatly affected by the fragmentation of habitats that those with generalist traits and greater dispersal ability (With and Crist, 1995). Therefore, functional connectivity is a key factor for the survivorship of many habitat-specialist species (Stevens *et al*., 2006). Areas with low connectivity coupled with degraded habitat and intensive human land use can face extinction cascades, especially if key species or groups of species are lost (Fischer and Lindenmayer, 2007; Miyazono *et al*., 2010). Functional connectivity has also been shown to be a key determinant of animal distributions in heterogeneous landscapes (Crooks and Sanjayan 2006).

1.2.1 Differences from structural connectivity

Often, what is meant by the term 'connectivity' is structural connectivity (sometimes called physical connectivity), which simply implies that a connection exists (e.g. a fishway) and that habitats are both adjoining and adjacent (Van Looy *et al*., 2014). Functional connectivity, on the other hand, includes how organisms move among habitats within a landscape (Kadoya 2009; Van Looy *et al*., 2014) and how a landscape influences the movement of organisms (Taylor *et al*., 1993, Kadoya 2009). Structural connectivity accounts for habitat size and inter-habitat distance but does not consider any behavioural influences on organism movements (Dunning *et al*., 1992; Crooks and Sanjayan 2006). Generally, it is recognised that functional connectivity will more faithfully reflect organism spatial dynamics by taking into account their responses to different habitats and landscape between the habitats (With *et al*., 1997, Belisle, 2005). Therefore, functional connectivity more accurately accounts for biotic responses to habitat structure and composition (Belisle 2005). Structural connectivity is more commonly measured when compared to functional connectivity (Doerr *et al*., 2011).

1.2.2 Functional connectivity and its importance for habitat use and selection

Functional connectivity has been poorly studied compared with structural connectivity (Dancose *et al*., 2011). To address this limitation, we need to quantify habitat connectivity for species with different ecological needs, focusing on functional (as opposed to structural) connectivity and treating that connectivity as variable in both space and time (Mimet *et al*., 2013). While it is important to preserve key habitats, which is a current conservation target worldwide (and should continue to be), improvements in connections between these key habitats should also be considered a conservation priority (Martensen *et al*., 2008). This is because enhancing functional connectivity is beneficial to a wide range of species within a landscape (Martensen *et al*., 2008).

It has been shown in large terrestrial mammals (bison) that habitats with a larger overall size are typically selected over smaller habitats (Dancose *et al*., 2011). Organisms have observable tendencies to move to a given habitat dependent on its accessibility, size and intrinsic quality (Arthur *et al*., 1996, Rhodes *et al*., 2005, Martin *et al*., 2008). Often, organisms have also been shown to select habitats in which they can achieve the highest possible level of fitness (Morris and Davidson 2000, McLoughlin *et al*., 2006). However, organisms do not always use the shortest route to get to a new habitat (Dancose *et al*., 2011).

As may be expected, non-migratory organisms tend to transit to habitats closer to their current location than migratory organisms. This pattern has been shown in many vertebrates including: non-migratory birds (Lynch and Whigham 1984, Andersson and Bodin 2009); mammals (Rodriguez and Andren 1999, Dancose *et al*., 2011); and amphibians (Chan-McLeod and Moy 2007). Also as expected, suitable food resources within the next selected habitat have been shown to influence the destination of large mammals (Bison; Dancose *et al*., 2011, domestic sheep; Edwards *et al*., 1996 and elk; Wolf *et al*., 2009). Therefore, despite only limited studies focusing on functional connectivity the understanding of an organism's perceptions of- and behaviour within- an environment are important for the conservation of habitats.

1.2.3 Connectivity and habitat use in a freshwater environment

Studies of habitat patch use and functional connectivity for species that do not undergo large scale migrations are becoming more common in science, including for large-bodied terrestrial mammals (bison; Dancose *et al.,* 2011), marine species (reef fish; Mumby and Hastings, 2008; Turgeon *et al*., 2010) and birds (brown treecreeper; Doerr *et al.,* 2011). However, few studies have focused on freshwater ecosystems. In freshwater ecosystems, it is more common to assume that hydraulic connectivity (i.e. structural connectivity) equates to functional connectivity. This assumption, that when there is water connecting two habitats, fish (or other organisms) are able to use both, may not hold in all cases, particularly if flow rates, volumes or timing are inappropriate for the organisms involved (Lester *et al.*, 2011). Functional connectivity may also be dependent on an animal's ability to perceive and use pathways among different habitats as well as their movement among them (Kadoya, 2009) and may be interrupted by natural and/or artificial barriers (Lester *et al.*, 2011). These barriers can be physical (e.g. weirs), temporal (e.g. droughts) or seasonal (e.g. flooding not aligning with breeding seasons; Closs *et al.*, 2004). Investigation of functional connectivity in freshwater environments across different times and scales is needed to gain an understanding in aquatic biodiversity and should provide greater insight into ecosystem processes, functions and services when compared to structural connectivity studies (Ward *et al*., 1999). It has been suggested that when considering functional connectivity for fish that mobility of the fish species and water current be considered (Caldwell and Gergel, 2013). It is predicted that demersal fish will be more sensitive to habitat changes when compared with pelagic fish, as pelagic fish are typically more mobile and more easily able to relocate into areas of favourable habitat (Caldwell and Gergel, 2013). Decreases in connectivity have been linked to local extinctions of specialist fish taxa and to increases in generalist fish taxa in floodplain lakes (Miyazono *et al*., 2010).

1.2.4 Measures of fish functional connectivity

The movements of certain fish taxa may be measured easily however, determining how that movement affects assemblage composition and gene flow can be both costly and time consuming, depending on the taxa and methods chosen. There are three broad methods used when measuring connectivity for fish:

1. Physical tracking;
2. Genetically and chemically tracking; and
3. Modelling connectivity.

Physically tracking individuals is a useful tool for understanding connectivity in the short to medium term and can account for small to medium spatial scales depending on the method. Some of the methods for physically tracking individual fish include: calcien marking (e.g. Bice *et al.,* 2013); tagging (e.g. Kanno *et al*., 2014); and fish capture time series studies (e.g. Ferguson *et al.,* 2013). Physically tracking individuals can be an expensive method as, for example, individual tags can range in cost from less than $10 (PIT tags) to several hundred dollars (radio and acoustic tags) plus the additional cost of setting up arrays to detect the tags (Dotson and Kiefer, 2006).

Genetically and chemically tracking is another method used to assess connectivity over the short to medium term and is often conducted over medium to large scales where differences in genetic or chemical makeup can be detected. Some of the methods for assessing fish movements through time include: otolith microchemistry (e.g. Elsdon and Gillanders, 2003), stable isotope analysis (e.g. Cresson *et al.* 2014) and genetic assessments (e.g. Stevens, *et al*., 2006; Walter *et al*., 2011). These methods enable the determination of movements between water bodies: via primary production or trophic levels present at a particulate location (stable isotope analysis; Cresson *et al.* 2014); via trace element accumulation over time (otolith microchemistry; Elsdon *et al.* 2008); and via gene flow and genetic makeup across a landscape (genetic assessments; Stevens, *et al*., 2006). However, functional connectivity assessment by these methods has rarely been validated by *in-situ* observations (Stevens, *et al*., 2006).

Connectivity modelling has also been used to assess connectivity and can be done over a short or long term and at small or large scales depending on the method used. Some of the methods for modelling connectivity for fish include: network analysis (McKay *et al.,* 2013); multimetric indices (Perez-Dominguez *et al.,* 2012; Schoolmaster *et al.,* 2013; e.g. index of river connectivity; Sola *et al.,* 2011; estuarine multi-metric fish index; Harrison and Kelly, 2013); and particle tracking simulations (Ashford *et al.,* 2010). These methods are often based on the comparison of fish potential pathways, obstacles and fishway characteristics (Sola *et al.,* 2011). However, this method of assessing functional connectivity requires knowledge of the capabilities (e.g. swimming ability) of the fish present within a system and their movement characteristics among habitats (Sola *et al.,* 2011).

Currently, it appears that that no single method is able to accurately determine functional connectivity for all fish species at different spatio-temporal scales. Only through using a combination of these methods and selecting methods that assess the correct spatio-temporal scale for the fish species present within a system can we gain a meaningful understanding of fish functional connectivity.

1.3 Semi-arid aquatic ecosystems

Semi-arid and arid regions (25-500 mm year^{-1} rainfall) cover approximately one-third of the world's land area and are home to more than 400 million people (Williams, 1999). The number of people living in these areas is increasing and therefore water is under increased demand due to human pressures (Williams, 1999). In Australia, there has been rapid development of water resource-infrastructure in semi-arid and arid regions which has altered the flow regime and ecology of the large rivers running in these regions (Sheldon *et al*., 2002). Current climate change scenarios forecast less precipitation and longer dry periods, coupled with an amplification of summer extreme weather events (Watterson *et al.,* 2008; Collins *et al*., 2013). There are limited ecological data of the likely response of flora and fauna to concomitant changes in flow regime (Sheldon *et al*., 2002). Furthermore, the ecological significance of connectivity is poorly understood in semi-arid regions especially in systems which experience sporadic flooding (Sheldon *et al*., 2002). Thus, studies focused on biotic responses to varied flow will be important for future management of these systems.

1.4 Study region: the Lower Lakes

The Lower Lakes (Lakes Alexandrina and Albert), in semi-arid south-eastern Australia, lie at the terminus of the Murray-Darling River. The Murray-Darling Basin (MDB) is the largest drainage system in Australia and comprises an area of around 1 million km^2 and supports a diverse assemblage of flora and fauna (CSIRO, 2008). In South Australian, the River Murray is the primary river and is highly valued economically, socially, culturally and ecologically (CSIRO, 2008). The Murray-Darling is a highly modified river system and modification of this river system began in the 1850s and continues to present day. The Murray-Darling river system is subject to significant water extraction for irrigation and urban use and since the installation of locks, dams and barrages only approximately 40% of natural mean river discharge reaches the sea (Walker, 2006; CSIRO, 2008). In addition, four large reservoirs have been constructed along the river system for the purpose of water storage and flood mitigation. Regulation of river flows has also increased closure of the estuary mouth from ~1% of the time without development to ~40% under current conditions (CSIRO, 2008), creating additional barriers for fish movement. Even with increases in environmental flow allocations there is unlikely to be enough water to provide optimal water levels to all ecosystems and this effect will be amplified during drought years (Ye *et al*., 2014). Therefore, environmental flows need to be prioritised based on which ecosystems need to receive water, how much they need and when, so as to maximise ecological outcomes (Lester *et al*., 2011; Ye *et al*., 2014).

The River Murray enters the top of Lake Alexandrina and water flows from Lake Alexandrina to either Lake Albert though a narrow channel or thorough the Goolwa channel to the Murray Mouth and Coorong estuary (Phillips and Muller, 2006). Lake Alexandrina, the larger of the two lakes, is approximately 650km^2 and contains just over 1600 GL when full whereas Lake Albert is approximately 230 km^2 and contains just over 280 GL when full. Both lakes are dominated by sandy benthos with patches of more-complex habitat most commonly found in the fringing areas. A series of man-made barrages form a biological and physical barrier between the Lakes and the Coorong and Southern Ocean. These barrages were constructed to prevent saltwater intrusion into the Lower Lakes in the 1930s (Maheshwari *et al*., 1995). Recent drought ('Millennium Drought'; LeBlanc *et al*., 2012) caused the Lower Lakes to fall below sea level in 2008 for the first time in recorded history (MDBA, 2014) and post-drought flooding in 2010/11 facilitated a re-structuring and re-population of macrophyte communities (i.e. potential fish habitat) as well as increased large-bodied fish spawning and recruitment (Ye *et al*., 2014). The impact of increased large-bodied fish populations on small-bodied fish is, to my knowledge, unknown.

1.4.1 Lower Lake hydrology and environmental flows

Environmental flows, intended to sustain aquatic ecosystems, are usually designed assuming that hydrological connectivity equals functional connectivity. That is, we assume that physical connection is the same as providing for the movement of individuals, groups or whole populations within and the aquatic landscape. But, this may not be the case if flow rates, volumes or timing are inappropriate (Lester *et al*., 2011). Under low-flow conditions, such as those experienced during the

Millennium Drought (<15000 ML day^{-1}), the River Murray can be characterised by a series of lentic pool habitats which contrasts the river's highly variable, lotic historical form (Walker, 2006). Flooding within the Lower Lakes and greater MDB in 2010/11 caused an increase in primary production, improved both lateral and longitudinal connectivity, increased lateral bank recharge, re-structured the aquatic plant communities, increased plant recruitment and led to increased large-bodied fish spawning, recruitment and movement (Ye *et al*., 2014). Increasing flows to facilitate large-scale connectivity is thought to be important for the health of the MDB as a whole (Ye *et al*., 2014). Maintaining lateral connectivity is thought to be critical, especially when connecting productive wetland and floodplain habitats (Ye *et al*., 2014). Returning to this highly variable regime, with a mixture of flow sizes, would likely benefit many of the functional groups within the MDB and potentially even restore and maintain aquatic ecosystems (Ye *et al*., 2014).

Water levels within Lakes Alexandrina and Albert have historically been static and small changes in lake level can influence things like fringing vegetation availability. The average lake level over the last three years has been approximately +0.7 m Australian Height Datum (AHD), which approximates mean sea level. During this time, Lake Alexandrina and Albert have varied between +0.5 to +0.88 m AHD and +0.47 to +0.9 m AHD, respectively. Lake levels below +0.35 m AHD in Lake Alexandrina disconnect the majority of littoral vegetation and wetlands (Lester *et al*., 2011). At lake levels from +0.35 to +0.7 m AHD, there is a decrease in exposed mudflats and an increase in the amount of littoral vegetation inundated, until the vast majority of littoral vegetation is inundated at levels at or above +0.7 m AHD. For levels higher than +0.83 m AHD, overland flows across Hindmarsh Island occur and the majority of floodplain habitat is connected (Lester *et al*., 2011). During the Millennium Drought in 2008, water levels in the Lower Lakes fell below sea level (MDBA 2014) which caused floodplain and fringing vegetation to become desiccated (Nicol, 2010) and exposed large areas of acid sulphate soil within the lakes (Simpson *et al*., 2010).

1.5 Analogous semi-arid aquatic ecosystems

There are a number of semi-arid aquatic ecosystems worldwide but two of the ecosystems most comparable to the Lower Lakes are the Colorado River Delta, USA and St. Lucia Estuary, South Africa. These two aquatic ecosystems face similar alterations as the MDB such as water extraction for irrigation and urban uses, as well as damming for power generation and water storage (Vardy *et al.,* 2001). Often these intensive uses have resulted in significant departures from natural conditions in terms of biodiversity and hydrology (United States Department of the interior, 2005; Smith-Adao, *et al*., 2011). These systems also face the same challenges including identifying conservation priority areas, supplying enough water for both human and ecosystem use and the rehabilitation of degraded areas (Smith-Adao, *et al*., 2011). For the Colorado River, only 10% of water historically reaches the USA-Mexico border due to political agreements (Vardy *et al.,* 2001; United States Department of the Interior, 2005), of which none typically reaches the sea, with water specifically being released for the environment for the first time in 2014 (Buono and Eckstein, 2014). Supplying enough water for both humans and the environment is difficult to achieve and a recent survey in the St. Lucia Estuary found that one river reach represented a possible win-win situation for both the environment and humans (Smith-Adao, *et al*., 2011). Currently, the small-bodied fish within these systems are understudied and there have been no studies, to my knowledge, on functional connectivity on fish within the Colorado River Delta or St. Lucia Estuary. Therefore, there is the potential to apply any knowledge gained from this research to these systems and test the findings across multiple semi-arid aquatic ecosystems.

1.6 The small-bodied fish of the Lower Lakes

There have been around 80 species of fish recorded in the Coorong and Lower Lakes (Higham *et al*., 2002). For these fish species that have historically been present in the Coorong and Lower Lakes, the extent of knowledge and known occurrence of these species is spatio-temporally variable (Jennings *et al*., 2008). Seventeen fish are considered indicator species of environmental change in the South Australian MDB due to their range of habitats and life histories. Of these, ten have significant knowledge gaps about their use of environmental flows, habitat and connectivity (Lester *et al*., 2011). The small-bodied fish in the Lower Lakes have largely similar life histories and can be broken up into two broad groups: 1) those that are demersal and typically guard their eggs once spawned in vegetated habitats and 2) those that are pelagic, are thought to spawn in vegetation and don't exhibit parental care of their eggs (table 1.). The small-bodied fish in the Lower Lakes also have similar feeding groups being either omnivorous or carnivorous (Table 1) and in most cases depending on macroinvertebrates and zooplankton at a primary food source (Bice, 2010).

Native small-bodied fish (e.g. fish with a maximum adult length of <200 mm as opposed to the juveniles of large-bodied fish) are important in the Lower Lakes of the River Murray for numerous reasons. Small-bodied fish within the Lower Lakes are thought to provide an essential food resource for larger vertebrates such as fish and birds. The iconic large-bodied predatory fish species such as Murray cod *Maccullochella peelii* and silver perch *Bidyanus bidyanus* are reliant on small-bodied fish within Lakes Alexandrina and Albert as a food resource (*Fisheries Management Act* 2007, SA). Both of these large-bodied fish are listed as rare and vulnerable species (*Environment Protection and Biodiversity Conservation (EPBC) Act* 1999) respectively and are protected under South Australian law. The Lower Lakes are a part of a Ramsar-listed wetland of international importance, with an important breeding population of fairy terns *Sterna nereis.* Some small-bodied fish such as the small-mouth hardyhead *Atherinosoma microstoma* (table 1) are considered key species for their role in providing a food resource for the fairy tern populations.

The impacts of drought and human regulation have particularly affected some now-rare iconic small-bodied fish species such as the southern pygmy perch *Nannoperca australis* and Yarra pygmy perch *Nannoperca obscura* (table 1 which have been listed as endangered and protected under South Australian law (*Fisheries Management Act* 2007, SA). Also of the other rare small-bodied fish species known to historically exist within the Lower Lakes, two *N. obscura* and the Murray hardyhead *Craterocephalus fluviatilis* are listed on the *EPBC Act,* and four *N. obscura*, *C. fluviatilis*, *N. australis* and *M. adspersa* are protected under the *Fisheries Management Act* 2007, SA. Despite more stable conditions within the Lower Lakes, i.e. post drought and flood, the small-bodied fish assemblage within the Lower Lakes have not shown the same populations increases as the large-bodied fish (Wedderburn *et al*., 2014; Ye *et al*., 2014). It is thought that the combination of drought followed by flooding indirectly affected the small-bodied fish by changing the macrophyte cover and resulted in the loss of much of the submerged macrophytes (Bice *et al*., 2013; Ye *et al*., 2014). In contrast, the large-bodied fish has experienced increased spawning and recruiting as a result of the flooding due to the direct influence of the increased flow on these life history processes (Zampatti and Leigh 2013; Ye *et al*., 2014). Overall, the post-drought flooding resulted in a significant change to both the large- and small-bodied fish assemblages which included decreased abundances of carp gudgeon complex *Hypseleotris* spp., flat-headed gudgeon *Philypnodon grandiceps*, dwarf flat-headed gudgeon *Philypnodon macrostomus*, mosquitofish *Gambusia holbrooki* and increased abundance of large-bodied fish especially common carp *Cyprinus carpio* (Ye *et al*., 2014).

Table 1. Small-bodied freshwater fish of the Lower Lakes with an assessment of commonality, likely habitats, feeding group and spawning type (adapted from McNeil and Hammer, 2007; Bice, 2010). Spawning type: 1. Typically demersal fish that lay adhesive eggs on vegetation, structure or in a nest that the male will typically guard. They exhibit protracted serial or repeat spawning over spring and summer; 2. Typically pelagic fish that lay adhesive eggs attached to vegetation or structure with no parental care. Individual will undergo either protracted, serial or repeat spawning over spring and summer; 3. Pelagic exotic fish that is live-bearing over spring and summer; and 4. Typically demersal fish that spawns in marine environment. * spawns over a short period.

Small-bodied fish:		**Commonality:**	**Likely habitat:**	**Feeding group:**	**Spawning type:**
Small-mouthed hardyhead	*Atherinosoma microstoma*	Very common	Habitat generalist	Carnivore	2
Flat-headed gudgeon	*Philypnodon grandiceps*	Very common	Muddy substrates and macrophytes	Opportunistic carnivore	1
Australian smelt	*Retropinna semoni*	Very common	Slow-flowing water	Opportunistic carnivore	2
Eastern gambusia	*Gambusia holbrooki*	Very common	Littoral habitats and macrophytes	Carnivore	3
Bluespot goby	*Pseudogobius olorum*	Common	Muddy and rocky substrates, macrophytes and shallow water	Omnivore	1
Carp gudgeon complex	*Hypseleotris spp.*	Common	Slow-flowing water and macrophytes	Generalist omnivore	1
Common Galaxias	*Galaxias maculatus*	Common	Habitat generalist	Opportunistic carnivore	2*
Lagoon goby	*Tasmanogobius lasti*	Common	Muddy and rocky substrates, macrophytes and shallow water	Opportunistic carnivore	Unknown
Dwarf flat headed gudgeon	*Philypnodon macrostomus*	Moderately Common	Muddy substrates and macrophytes	Carnivore	Likely 1
Goldfish	*Carassius auratus*	Common	Macrophytes	Omnivore	2
Unspecked hardyhead	*Craterocephalus stercusmuscarum*	Moderately Common	Littoral habitats	Carnivore	2
Congolli	*Pseudaphritis urvillii*	Moderately Common	Complex structures with Sandy and muddy substrates	Benthic carnivore	4
Tamar goby	*Afurcagobius tamarensis*	Moderately Common	Slow flowing water with Sandy and muddy substrates	Carnivore	Unknown
Bridled goby	*Arenigobius bifrenatus*	Un-common	Complex structures with Sandy and muddy substrates	Opportunistic carnivore	1

Small-bodied fish:		Commonality:	Likely habitat:	Feeding group:	Spawning type:
Southern pygmy perch	*Nannoperca australis*	Rare	Shallow water and macrophytes	Carnivore	2*
Murray hardyhead	*Craterocephalus fluviatilis*	Rare	Slow-flowing water and macrophytes	Omnivore	2*
Yarra pygmy perch	*Nannoperca obscura*	Rare	Shallow water and macrophytes	Carnivore	2*
Murray rainbow fish	*Melanotaenia fluviatilis*	Rare (1986 - last sighting)	Slow-flowing water and macrophytes	Opportunistic carnivore	2
Southern Purple-spotted gudgeon	*Mogurnda adspersa*	Thought to be locally extinct	Shallow water and macrophytes	Benthic carnivore	1

1.6.1 Small-bodied fish habitat

Many fish species use different habitats during different life stages (Closs *et al*., 2004). Water levels within the Lower Lakes have the ability to influence the availability of habitat for small-bodied fish species in the Lower Lakes by connecting and disconnecting areas of habitat and changing habitat diversity (Whitfield, 2005). There are significant knowledge gaps concerning small-bodied fish habitat use, recruitment and population dynamics (age and size structure), as well as connectivity among habitats both within the Lower Lakes and between the Lower Lakes and the surrounding aquatic environments including the river and Coorong (Lester *et al*., 2011). Proximity to habitats is expected to be important for small-bodied fish and it is expected that the abundance and diversity of small-bodied fish within a given area will decline with distance between habitats (Olds *et al*., 2013).

Previous research has investigated the broad-scale habitat use of fish assemblages for a number of broad habitat types (natural channels, artificial channels, lake edges and wetlands; Wedderburn *et al*., 2012; Wedderburn *et al.*, 2014). However, these studies do not focus on small-bodied fish specifically or the small-scale habitats that they use and therefore it is difficult to conclude what small-scale habitat is used by small-bodied fish. The majority of the small-bodied fish recorded within the Lower Lakes have been shown to have some association with macrophyte habitats on a broad scale (Bice, 2010; Wedderburn *et al.*, 2012*;* Wedderburn *et al*., 2014), but the, the specific small scale habitat needs and uses of many of the small-bodied fish in the Lower Lakes are unknown.

Artificial structures are an understudied potential habitat type for small-bodied fish. In one study in the USA, 70 % of the relatively small fish (pumpkinseed fish *Lepomis gibbosus* ~150-200 mm and shiners *Notemigonus crysoleucas* ~75-125 mm) captured were among artificial cover (cinder blocks, tire bundles and brush bundles), 29 % among natural weed beds and 1 % in areas without cover (Moring & Nicholson, 1994). Furthermore, the number of fish associated with artificial habitat was higher at night (Moring & Nicholson, 1994). Small cichlids (e.g. *Geophagus brasiliensis*; <150 mm) in Central America have also been associated with artificial structures (Santos *et al.*, 2008). Other studies in freshwater have focused on artificial rock reefs (e.g. Creque *et al.,* 2006; Daugherty *et al.,* 2014) and have found significant fish associations. There have been no studies on Australian freshwater small-bodied fish using artificial structures, in particular jetties, to my knowledge. Therefore, small-bodied fish using artificial structures such as jetties will be explored further.

1.6.2 What are the Lake level needs of a small-bodied fish

Lake level is thought to be the overarching driver of fish assemblages within the Lower Lakes and influences other factors such as physicochemical characteristics and connectivity (Bice and Ye, 2009; Bice 2010). The effects of flow, which lead to changing lake levels in the Lower Lakes, have been shown to affect ecosystem structure and function which, in turn, influences the abundance and distribution of aquatic biota, including fish (Poff and Allan 1995; Sparks *et al*., 1998). It is thought that increased lake levels would promote longitudinal and lateral connectivity between habitats by facilitating fish movement (Reynolds, 1983), dispersal (Dudley and Platania, 2007) and by inundating habitat that is unavailable at low lake levels (Junk *et al*., 1989). Previous studies have also demonstrated the negative effects that reduced freshwater input can have on estuarine fish which are commonly found in the Lower Lakes (e.g. *A. microstoma*; Whitfield 2005; Bice; 2010; Zampatti *et al*., 2010). Even small flows (<50 ML day^{-1}) act as cues for estuarine biota and promote diversity and recruitment in estuarine fish assemblages which can lead to increase in the estuarine fish inhabiting the Lower Lakes (Bice, 2010; Zampatti *et al*., 2010). Furthermore, in the MDB, significant declines in fish assemblages have been attributed to the effects of river regulation which has caused reduction in the water available for the environment (Cadwallader 1978; MDBC, 2004). Moreover, changes in water availability can influence productivity within an aquatic environment and this may influence food availability (Poff *et al*., 1997). A combination of flows into the Lower Lakes (i.e. increasing lake levels), water levels within the Lakes (i.e. to inundate or disconnect fringing habitat) and flow out of the lakes (i.e. which can promote estuarine fish recruitment) all influence fish assemblages within the Lower Lakes (Ye *et al*., 2014).

1.7 Conclusion

Ecosystem functional assessments allow for a greater understanding of the ecosystem when compared to ecosystem process assessments, as they more wholly account for ecological properties (Jax, 2005). Functional connectivity is an important ecosystem function (Martensen *et al*., 2008) but has been rarely studied in freshwater environments and not for small-bodied fish within the Lower Lakes. The Lower Lakes will act as an ideal case study for small-bodied fish habitat use and functional connectivity under differing lake level conditions which are current knowledge gaps (Lester *et al*., 2011). The results of this research may also be applicable to other semi-arid aquatic ecosystems such as the Colorado River Delta and St. Lucia Estuary. Developing a greater functional understanding of the small-bodied fish assemblage within the Ramsar-listed Lower Lakes may also be important for the conservation of multiple species including both small- and large-bodied fish and birds as well as the functioning of the overall ecosystem.

Section 2. Research questions, plans and current status

2.1 Overall project design

This PhD research will be conducted within the Lower Lakes (Alexandrina and Albert), River Murray, South Australia. These lakes will be used as a case study region, analogous to other semi-arid lake ecosystems worldwide. There are currently significant knowledge gaps in our understanding of how structural connectivity can be used to assume functional connectivity, particularly for small-bodied native fish. There are also knowledge gaps relating to small-scale habitat use by small-bodied fish within the Lower Lakes for food, shelter or breeding. In order to better understand the habitat use and connections between different habitat types, a functional approach is needed. Therefore, the overall objectives of this PhD research are:

1. To document inter- and intra-lake habitat use and functional connectivity for small-bodied native fish, particularly focusing on how lake levels influence habitat availability;
2. To develop habitat-envelope models for selected small-bodied fish based on their habitat use and functional connectivity; and
3. To identify whether small-bodied fish are using different microhabitats as a food source, for shelter and identify the range over which they are moving.

These objectives will be achieved through four separate projects, starting with an assessment of the impact of lake levels on habitat use for small-bodied fish species in the Lower Lakes (Project 1). The data collected on fish habitat use from Project 1, along with data from other sources, will then be used to develop habitat-envelope models for small-bodied fish. The models will be based on how habitat use is influenced by parameters including connectivity, lake water volume and small-scale habitat characteristics that are identified in Project 1 as significant (Project 2). A suite of complementary methods will also be employed to quantify movement and connectivity between habitat patches of different habitat types. These will include the use of food-web studies (including both stomach content and stable isotope analyses) to identify differences in the activities (e.g. breeding, feeding, shelter) that small-bodied fish may undertake in different habitat types (Project 3) and the use of otolith microchemistry to track past movements and natal origins of individuals (Project 4).

2.2 Project 1: Quantifying changes in the occurrence of small-bodied fish in different habitats depending on water level

Aim: To quantify small-bodied fish assemblages within four fringing habitat types in the Lower Lakes focusing on how lake levels influence the availability of the habitat used. Previous research has investigated the use of broad-scale habitat types (i.e. natural channels, artificial channels, lake edges and wetlands; Wedderburn *et al.* 2012; Wedderburn *et al.*, 2014) by small-bodied fish and recommended that finer-scale microhabitat use be investigated. Thus, this project will investigate microhabitat use by small-bodied fish within the lake edge habitat type.

Methodology: The target fish for the initial project will be small-bodied native fish that exhibit protracted spawning over the spring/summer period (Bice, 2010). These fish will likely form the bulk of the catch however, analysis will include all small-bodied fish captured. Sampling will occur on six occasions over a 2-year period at a range of lake levels. November to March in 2014/15 and 2015/16 will be sampled to coincide with the period in which fish will be using the habitat most (during the breeding/spawning season, as well as for feeding and/or shelter; Bice, 2010). Typical lake levels range between +0.5 and +0.8 m AHD according my analysis of lake level over the past three years (2011-2013). I aim to sample at either end of this range (i.e. +0.5m AHD and +0.8m AHD) and will then allocate the remaining four sampling periods so as to span the continuum (i.e. between +0.5 and +0.8 m AHD). I will also sample opportunistically if any extreme events occur (i.e. lake levels below +0.5 m AHD or above +0.8 m AHD). Seven replicates of each habitat type will be sampled based on a power analysis conducted using the data collected for lake edge catch from Wedderburn & Barnes (2012). Four microhabitat types will be targeted, including: Emergent and submerged vegetation complex; Emergent dominant vegetation (emergent vegetation where it occurs with no or minimal submerged vegetation); artificial structures (jetties); and bare sediment (areas with <10 % occurrence of vegetation). Each site will be sampled using three small-mesh single-wing fyke nets and 12 box traps set overnight (dusk to dawn). Fyke nets and box traps are a commonly-used method for capturing small-bodied fish and have previously been used effectively within the Lower Lakes (e.g. Cheshire *et al.,* 2013; Wedderburn *et al.*, 2014; Thwaites and Fredberg, 2014). Seine net hauls were considered for this project but were not selected due to the potential damage to vegetation. Furthermore, fyke nets have been shown to be equally as effective as seine net hauls in small-bodied fish capture in the lower River Murray (Smith *et al.*, 2009). Proximity to the next available habitat (other than bare sediment) will be measured as a surrogate for connectivity (Olds *et al*., 2013). At each site, a habitat survey will be undertaken to quantify the microhabitat available and will include: three habitat transects; a visual assessment of habitat present; an assessment of habitat complexity and volume displacement; macrophyte percent cover; canopy cover; depth; distance from bank; sediment type; sediment size and pH of the pore water. All sites will also be surveyed for any artificial structures. For jetty structures, the number of pylons, their size and level of colonisation by biofilms or other biota will be estimated. Aerial photos of each artificial structure will also be assessed to determine the footprint of the structure. A suite of water quality variables will also be measured at each site including dissolved oxygen, turbidity, pH, specific electrical conductivity (as a surrogate for salinity), temperature, and nutrient concentrations, including ammonia.

Expected outcome: I hypothesize that the emergent and submerged vegetation complex habitat will support more diverse and abundant small-bodied fish assemblages, compared with artificial structures and bare sediment, because previous research suggests that these habitat types may be important refuge, feeding and breeding habitats for the majority of small-bodied fish within the Lakes (Bice, 2010). This project will determine what habitats are being utilised by small-bodied fish within the Lower Lakes depending on different lake levels. This knowledge may be used to develop appropriate management strategies to maintain appropriate water levels to ensure that suitable habitat is available at an appropriate time (i.e. during spring and summer for recruitment) for the small-bodied fish within the Lakes.

Status: Project design has been developed in association with the South Australian Research and Development Institute (SARDI), Department of Environment, Water and Natural Resources (DEWNR) and Flinders University and was presented at the Deakin HDR annual conference in October 2014. An ethics application is currently being reviewed by the Deakin University Ethics Committee and a ministerial exemption for the deployment of fyke nets and box traps is currently being sought from Primary Industries and Regions South Australia. The project's field safety assessment is currently being completed, and the first sampling trips scheduled for the 8th to the 20th of December 2014, with additional sampling periods to follow based on lake levels. Partial funding to conduct the field work was successfully sought from the Holsworth Wildlife Research Endowment ($1550).

2.3 Project 2: Habitat-envelope modelling for small-bodied fish depending on water level

Aim: To develop habitat-envelope models for small-bodied fish combining habitat use, type and availability at different lake level to predict the likely occurrence of small-bodied fish at a given site.

Methodology: Small-bodied fish assemblages, microhabitat type and use, lake level, proximity to the next available habitat (as a surrogate for connectivity) and other small-scale habitat characteristics measured in Project 1 will be used to model small-bodied fish habitat suitability and likelihood of presence of target species in given locations at various lake levels. This will be done using specialized computer modeling software, HyperNiche (MjM software, 2004). HyperNiche is a non-parametric multiplicative regression habitat modeling tool which will be used to estimate the likelihood that fish species will occur in relation to certain key habitat and environmental parameters (McCune, 2006), focusing on habitat availability and lake levels in particular. Habitat-envelope models will be based on the methodology developed for the region by Linn (2014). Predictive models will be based on the results from the initial sampling undertaken as a part of Project 1. Predictions made by the models will be tested and refined based on the on-going results of Project 1. The ultimate goal of this project is to develop robust models that can replace the need to extensively sample the small-bodied fish assemblage at each site and rely on a more cost and time effective and efficient habitat assessment (i.e. the ability to predict the likely fish population based on available habitat at a given location and lake level). This will prove valuable for use in management assessment of competing alternatives for environmental flow delivery and other required actions such as the need for re-vegetation or habitat conservation in key areas of the Lakes.

Expected outcome: I hypothesis that, at low lake levels, habitat suitability will be greatly reduced when compared to high lake levels and that small-bodied fish will be predicted to be present ay fewer locations at low lake levels compared to high lake levels. Project 2 will build on the findings of Project 1 and maybe important for the future management of Lakes Alexandrina and Albert, allowing managers to predict the influence of lake level on the current small-bodied fish assemblage without the need to extensively sample fish populations. This project will also allow managers to predict the effect of future lake level changes on small-bodied fish species allowing more informed decisions to be made regarding the management of the lake volume and conservation of habitat areas. This project should also highlight any key habitat areas within the Lakes that should be enhanced, maintained or restored and the importance of maintaining connectivity among habitats to benefit a wide range of fish.

Status: Modelling will occur after the collection of data from the first sampling event from Project 1 and the best models will be validated during future sampling events and refined if required.

2.4 Project 3: Trophic structure within two highly-regulated lakes based on four abundant small-bodied native fish with opposing life histories

Aim: To investigate the food-web structure associated with different habitat types to identify whether fish are using different habitats as food resources.

Methodology: The most abundant macrophyte species, epiphytes and invertebrates, along with four target small-bodied native fish species, flat-headed gudgeon *Philypnodon grandiceps*, blue-spot goby *Pseudogobius olorum*, smallmouth hardyhead *Atherinosoma microstoma* and Australian smelt *Retropinna semoni,* will be sampled from various habitat patches within the Lakes. These four fish species were selected represent the two most common life histories (i.e. two are pelagic and the other two demersal) that are each representative of multiple species and have been historically abundant within the Lower Lakes (Bice, 2010). From each sample, stable isotope signatures of carbon and nitrogen will be measured to identify the likely primary production source (based on carbon isotopes) and trophic level (based on nitrogen isotopes; Cresson *et al.,* 2014) The target fish species will also be euthanized and dissected for stomach content to determine the dietary structure of these fish, a process that is complementary to isotope analyses (Cresson *et al.,* 2014). This will be matched against potential food items within habitat patches to determine whether they are used as a food source and whether movements between different habitats occur for feeding (i.e. if there are spatial differences in the availability of particular food items).

Expected outcome: I hypothesis that *P. grandiceps* and *P. olorum,* which are demersal fish, will have stronger associations with the habitat they are found within than the pelagic *A. microstoma* and *R. semoni*. Furthermore, stable isotope signatures and stomach contents of the *P. grandiceps* and *P. olorum* will more closely match the habitats from which they came than those of *A. microstoma* and *R. semoni*. This project will result in a comprehensive picture of the habitat association, differential use and functional connectivity among a range of microhabitat types, including open water, fringing vegetation and artificial structures for two abundant small-bodied native fish species with opposing life histories.

Status: Project design in development, ethics approval for this study will be sought in early 2015, funding successfully sought from the Holsworth Wildlife Research Endowment to conduct the stable isotope analysis ($5250).

2.5 Project 4: Movement and natal origin of four abundant small-bodied native fish with opposing life histories

Aim: To describe the movement and natal origin of the *P. grandiceps*, *P. olorum*, *A. microstoma* and *R. semoni* using otolith microchemistry. Furthermore, I will also quantify the range over which fish move so as to access different habitat patches to fulfil various life-history needs within and between the Lower Lakes to assess connections among the habitat patches.

Methodology: The otolith (ear bone) from the fish euthanized during Project 3 will be removed and undergo Laser Ablation Inductively Coupled Plasma Mass Spectrometer (LA-ICP-MS; Elsdon and Gillanders, 2003). Otolith chemistry enables the estimation of past environmental history of the fish (i.e. movements) and connections among habitats or at broader spatial scales, as well as an assessment of the natal origins of the four target fish species sampled based on trace element accumulation and stable isotopes (Elsdon *et al.,* 2008). Essentially, the otolith acts similarly to tree rings: as the fish grows so does the otolith and as the otolith grows it incorporates trace elements and different stable isotopes from the water in which the fish lives (Elsdon *et al.,* 2008). The elemental and isotopic relationships uncovered via LA-ICP-MS can give an environmental history of the individual fish from its early life to when it was captured (Gillanders and Munro, 2009). The edge of the otolith thought to represent the most recent growth and representative of environmental conditions near where the fish was captured (Gillanders and Munro, 2009). Otolith chemistry has been conducted on *A. microstoma* captured in the Coorong looking into the effects of salinity (Gillanders and Munro, 2009) but did not investigate *A. microstoma* within the Lower Lakes.

Expected outcome: This project will enhance our ecological understanding of the breeding habitat needs of the four target small-bodied fish species that inhabit Lower Lakes and potentially highlight the importance of connection between the different habitats within the lakes.

Status: Methodology is currently under development.

2.6 Overall expected outcome:

The overall result of this PhD research will be a comprehensive picture of the habitat association, differential use of, and functional connectivity among a range of fringing habitat types. This project will also enhance our ecological understanding of the feeding and shelter habitat needs of the small-bodied fish species that inhibit the Lower Lakes. In particular, understanding their use of habitats under differing lake levels will highlight habitats that need to be protected and conserved, particularly during dry periods. This knowledge may be used to develop appropriate management strategies to maintain appropriate water levels to ensure that suitable habitat is available for the recruitment and maintenance of small-bodied fish populations within the Lower Lakes. This project may also provide insight into why, despite the breaking of the Millennium Drought with flooding in 2010/11, the once-abundant and diverse assemblage of native small-bodied fish have not shown signs of recovery in recent years (Wedderburn *et al.*, 2014; Ye *et al*., 2014). Protection and conservation of the small-bodied fish will help maintain both iconic large-bodied fish populations (e.g. *Maccullochella peelii*, Murray cod and *Bidyanus bidyanus*, silver perch) and bird communities (e.g. *Sterna nereis*, fairy terns) within the Ramsar-listed Lower Lakes by safe-guarding an essential food resource (i.e. the small-bodied fish). This food resource is vital within the Lower lakes and some small-bodied fish, such as *A. microstoma*, are considered key species in the Lower Lakes. Furthermore, this project will be valuable in improving environmental flow delivery to achieve maximum ecological outcomes by directly linking flow, lake level and habitat provision. This project will also form part of a larger study investigating the impact of environmental flows on ecological functions. The larger study aims to identify indicators of ecological functions for semi-arid aquatic ecosystems in Australia and the USA/Mexico and is being undertaken in collaboration with Deakin University, Flinders University, University of Liverpool (UK), University of Arizona (USA), DEWNR, the Murray-Darling Basin Authority, and SARDI.

Section 3. Timetable

3.1 Timetable/progress to date

The following is the proposed schedule and timeline for the PhD candidature of Bryce Halliday at Deakin University. This timeline is flexible and will be adjusted throughout the candidature as required until final thesis is completed in early 2017. Project 1 sampling will occur throughout the candidature based on variations in lake level over 2015 and 2016. HyperNiche modelling (project 2) will occur once sufficient data has been collected. Stomach content and stable isotope analyses (project 3) and otolith microchemistry (project 4) will occur once sufficient samples have been taken. Conferences have not been included on the timetable as exact conferences and dates are yet to be determined. Efforts to attend at least one international conference and several domestic conferences will occur throughout candidature. However, project 1 design and methodology has been presented at Deakin's Higher Degree by Research conference in October this year (2014). As a minimum I am hoping to publish four papers from my thesis (please see section 5 for details).

Candidature 31/3/2014- 31/3/2017

2014 Month	04	05	06	07	08	09	10	11	12
Identification of potential research questions/topics									
Literature review									
Summary of Responsibilities of Supervisors and PhD candidate									
Research questions/topics discussion with DEWNR									
Research questions/topics discussions with SARDI									
Workshops – start 1-4, 6 & confirm 1-3, Research integrity training									
Candidature agreement									
Refinement of research questions/topics									
Meeting with Karl Flessa from University of Arizona									
Holsworth wildlife endowment grant									
Study design									
Deakin Animal ethics -Animal ethics for project 1 and 2 Submitted 22/9/14 -Animal ethics pre-review corrections submitted 3/10/14									
South Australian Ministerial exemption for project 1 and 2									
HDF conference									
Confirmation -Confirmation document submission 14/11/14 -confirmation presentation 1/12/14									
Sampling gear acquired									
CIE grant									
Cultural approval for project 1									
Manuscript preparation for project 1									
Small boat handling course									
Sampling trip 1									
Holsworth progress report									

2015 Month	01	02	03	04	05	06	07	08	09	10	11	12
Animal ethics for project 3 and 4												
Cultural approval for project 3 and 4												
South Australian Ministerial exemption for projects 3 and 4												
Manuscript preparation for project 1												
Sampling trip 2 and 3												
Data analysis, Modelling and trophic structure analysis												
Manuscript preparation for projects 2- 3												
Holsworth grant round 2												
ESA grant												
Sampling trip 4 – 6												
Data analysis and model refinement												
Refining Manuscript for projects 1 and 2												
Holsworth progress report												

2016 Month	01	02	03	04	05	06	07	08	09	10	11	12
Sampling trip 4 – 6												
Data analysis and model refinement												
Refining Manuscript for projects 1 and 2												
Identify potential list of examiners												
Otolith microchemistry												
Manuscript preparation for projects 4												
All manuscripts submitted												
Thesis writing												
Holsworth final progress report												

2017 Month	01	02	03
Thesis writing -Compile publications/manuscripts into thesis structure -Write general intro/methods/discussion			
Notice of intent to submit thesis			

Section 4. Resources

4.1 Resources required for this project

Equipment and software: Field surveys will be conducted on six occasions and require the use of the School of Life & Environmental Science 4WD and punt to access the various sites. A total of 10 small-mesh single wing fyke nets and 40 box traps have been hired from Austral Research and Consulting for the fish surveys. Two YSI water quality multi-meters will be taken on each field trip to assess the physicochemical properties of the lake. Additional survey gear will include GPS, waterproof camera and measuring instruments e.g. tape measure and scales. Habitat complexity assessment, processing stable isotope samples, stomach content analysis and otolith extraction will occur in the freshwater ecology labs at Deakin University's Warrnambool campus. Stable isotope analysis (costed at $10/sample) and Laser Ablation Inductively Coupled Plasma Mass Spectrometer (costed at $2000/50 samples) will be out-sourced. The modelling program HyperNiche will be used for all predictive models and PRIMER 6 with PERMANOVA+ add on will be used for statistical analysis.

Grants: Multiple grants have/will be sought during candidature. So far, the Holsworth Wildlife Research Endowment grant ($7000) and publication incentive scheme ($500) have been successfully sought, with a CIE HDR additional funding application ($4000) currently in review. In 2015 the Holsworth Wildlife Research Endowment continuing grant ($7500), Ecological Society of Australia Student Research Award ($1500) and Ian Potter Foundation small grant (if continued in 2015, up to $15000) will be sought. Up to an additional $3000 will be sought from Deakin University HDR funding to attend a yet to be determined conference.

Qualifications: Currently the only additional qualification required is a boating licence with a course to be conducted on the 2nd and 3rd of December and additional boat handling training to be conducted following the course with Sean Blake.

Section 5. Publications

5.1 Publication plan

The goal is to publish one paper for each of the four projects within this PhD research. The final thesis will be prepared as per the thesis by publication guidelines with either manuscripts or published papers acting as chapters along with a general introduction, methods and discussion. Hydrobiologia will be the target journal for project one, Ecological Modelling for project two, Freshwater Biology for project three and Marine and Freshwater Research for project four. Manuscript preparation will predominantly be conducted during periods where sampling cannot be completed (i.e. April to October 2015 and 2016) with the aim to have all four papers completed and submitted by the start of December 2016. Producing as many publications as possible is a goal for Bryce Halliday and he has already co-authored two papers in 2014 (Wickson… Halliday *et al.,* 2014; Halliday *et al.,* In Review).

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